NOTE Clinical Pathology

Effects of Mycotoxins on Chemiluminescent Response and Cytokine mRNA Expression of Bovine Neutrophils

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ABSTRACT. The effects of aflatoxin B1 (AFB1), aflatoxin M1 (AFM1), deoxynivalenol (DON) and zearalenone (ZEA) on the viability, chemiluminescent (CL) response and expression of cytokine mRNA of bovine neutrophils (PMNs) were evaluated. The opsonized zymosan (OPZ)-stimulated CL response of PMNs was significantly (P<0.05) decreased by AFB1 (>50 μg/mL), AFM1 (>50 μg/mL) and ZEA (>50 μg/mL). The phorbol myristate acetate (PMA)-stimulated CL response PMNs was significantly (P<0.05) decreased by AFB1 (>0.5 μg/mL), AFM1 (>50 μg/mL), ZEA (>500 μg/mL) and DON (>5 μg/mL). Treatment with AFB1 resulted in reduction in the mRNA expression of interleukin-1β and tumor necrosis factor-α of PMNs stimulated with OPZ and PMA. These results suggest that these four mycotoxins have inhibitory effects on the function of bovine PMNs.

KEY WORDS: aflatoxin (B1, M1), bovine neutrophils, deoxynivalenol, zearalenone.

Mycotoxins are secondary metabolites produced by molds and are known to have effects on the immune system [3, 5, 15]. There have been many studies on the effects of mycotoxins on lymphocyte functions, and the inhibitory effects of mycotoxins on the proliferation of lymphocytes have been described [1, 2, 4, 19]. Enhancing and inhibitory effects of mycotoxins on the production of cytokines by lymphocytes have also been reported [6, 8, 11, 16]. However, the effects of mycotoxins, including aflatoxin B1 (AFB1), deoxynivalenol (DON) and zearalenone (ZEA) [12, 17, 18], on functions of PMNs have not been fully elucidated. In this study, we investigated the effects of AFB1, aflatoxin M1 (AFM1), DON and ZEA on the viability, CL response and cytokine mRNA expression of bovine PMNs.

Two clinically healthy Holstein steers, 3 years old, were used. Blood was collected from the jugular vein using a vacuum blood sampling tube containing heparin sodium (20 IU/mL of blood). PMNs were obtained by Ficoll-conray density gradient centrifugation followed by hypotonic hemolysis as previously described [14]. The resulting cell population comprised more than 95% neutrophils, as determined by Giemsa stain, and more than 99% of the cells were viable when assessed by trypan blue dye exclusion. PMNs were suspended in Hank’s balanced salt solution (HBSS, Nissui Pharmaceutical Co.) at the concentration of 3 × 10⁶ cells/mL [14].

The mycotoxins used were aflatoxin B1 (AFB1; Alexis), aflatoxin M1 (AFM1; Alexis), zearalenone (ZEA; LKT Laboratories) and deoxynivalenol (DON; LKT Laboratories Inc.). AFB1 and AFM1 were dissolved in 1 mg/mL of dimethyl sulfoxide and then serially diluted in HBSS at final concentrations of 1 μg/mL to 100 μg/mL and 100 μg/mL to 10 μg/mL, respectively. ZEA and DON were dissolved in 5 mg/mL of ethanol and serially diluted in HBSS at final concentrations of 100 μg/mL to 10 μg/mL. The isolated PMNs were stimulated with opsonized zymosan (OPZ; Sigma Chemical Co.) at a final concentration of 10 μg/mL or with phorbol 12-myristate 13-acetate (PMA; Sigma Chemical Co.) at a final concentration of 20 μg/mL. The viability of unstimulated and OPZ- and PMA-stimulated PMNs, either addition of mycotoxins or not, was evaluated by the trypan blue exclusion [9].

Two hundred μL of cell suspension (1 × 10⁶ cells/mL) was added into a 96-well microplate and 10 μL of each of the concentrations of mycotoxins (AFB1: 1 μg/mL to 100 ng/mL, AFM1: 100 μg/mL to 10 μg/mL, ZEA: 100 μg/mL to 10 μg/mL, DON: 100 μg/mL to 10 μL/mL) was added, respectively. After preincubation at 37 for 30 min, 10 μg luminol (Sigma Chemical Co.) at a final concentration of 0.5 μg/mL and 10 μL of each stimulant was added and then CL response was measured during at 37°C for 30 min using a chemiluminescent reader (Luminescencer JNR, ATTO Corp.).

After adding 50 μL of each concentration of AFB1 to 5 × 10⁶/mL of PMNs and incubated at 37°C for 30 min, each stimulant was added and incubated for a further 3 hr at 37°C. To evaluate mRNA expression of interleukin (IL)-1β and tumor necrosis factor (TNF)-α, cytokine real time-polymerase chain reaction (PCR) for PMNs stimulated with mycotoxins and stimulants was carried out according to the method described previously [13]. Values were evaluated using analysis of variance, and differences among groups were analyzed by Kruskal-Wallis test. Values of p<0.05 were regarded as significant.

The viabilities of PMNs incubated with mycotoxins, in the absence of stimulants, were 85.4 to 94.0%, and 81.6 to 88.7% and 78.5 to 91.3% in PMNs stimulated with OPZ and PMA, respectively, and their values were not significantly different. No significant unstimulated CL response in
PMNs incubated with AFB$_1$ was found in our study. This finding was similar to that in the report of Geissler and Henderson [7], who found that AFB$_1$ was unable to stimulate arachidonic acid metabolism.

The CL responses of OPZ-stimulated PMNs were significantly (p<0.05) decreased by AFB$_1$ at concentrations above 500 pg/ml, AFM$_1$ at concentrations above 50 pg/ml and ZEA at concentrations above 50 pg/ml (Fig. 1), compared to those without stimulants. An inhibitory effect on the CL response in PMNs incubated with ZEA was also detected. Although the mechanism by which ZEA inhibited the CL response in bovine PMNs remains unclear, the suppressed CL response may be associated with the inhibition of metabolic activity of PMNs. In the case of DON, there was a tendency for there to be an increment of the CL response when the concentration of DON was increased; however, the differences were not significant (Fig. 2). CL responses of PMA-stimulated PMNs were significantly (p<0.05) decreased by AFB$_1$ at concentrations above 0.5 pg/ml, AFM$_1$ at concentrations above 50 ng/ml, ZEA at concentrations above 500 pg/ml and DON at concentrations above 5 pg/ml compared to those without mycotoxins (Fig. 3).

Ubagai et al. [18] reported that AFB$_1$ had no effect on the CL response of human PMNs stimulated with PMA. This is inconsistent with our finding that the CL response of bovine PMNs stimulated with PMA was suppressed by AFB$_1$. Moreover, Ubagai et al. [18] reported that a lower concentration of AFB$_1$ increased the CL response; however, 50 pg/ml of AFB$_1$ suppressed the CL response of human PMNs stimulated with zymosan. Takayama et al. [17] determined the effects of DON on the CL responses of bovine and porcine PMNs, and found that DON treatment suppressed them by 42% and 35% at 10$^{-5}$ M, respectively, and even more at 10$^{-4}$ M. The dose-dependent CL response with increasing concentrations of DON was consistent with our present results. The CL response of DON-treated PMNs was not significantly changed by OPZ; however, it was significantly decreased by PMA. As the suppressive effect on the CL response was greater when cells were stimulated with PMA, DON may play a role in inhibiting the downstream of PKC in bovine PMNs. This suggested that the cytotoxicity of DON was not expressed by the reaction via cell surface receptors.

For the effects of mycotoxins on cytokine mRNA expression in bovine PMNs, inflammatory cytokines IL-1$\beta$ and TNF-$\alpha$ were selected for evaluating the mycotoxins interacting with inflammatory and immunological reactions. mRNA expression of IL-1$\beta$ and TNF-$\alpha$ in PMNs did not
change in the absence of stimulants; however, mRNA expression of both IL-1/β and TNF-α decreased in the presence of OPZ and PMA (Fig. 4). The results of the present study showed that AFB1 decreased the mRNA expression of IL-1/β and TNF-α, which was inconsistent with the results reported by Marin et al. [10] showing that AFB1 increased the mRNA expression of pro-inflammatory cytokines and decreased the mRNA expression of anti-inflammatory cytokines in piglets. The reason for the difference in cytokine mRNA expressions in PMNS remains to be clarified, it may be associated with various factors such as species, type of mycotoxins and stimulants. Further studies are required to elucidate such effects on inflammatory and immunological reactions.

This study demonstrates that the CL response and mRNA expression in AFB1, AFM1, ZEA and DON -incubated PMNs were more decreased when the cells were stimulated with OPZ and PMA than in those without stimulation. These results may be associated with the uptake of mycotoxins during cell activation and differences in signal transduction.

REFERENCES


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