# Effect of Aflatoxin $B_1$ on the Function of Peritoneal Macrophage from Mule Duck

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**ABSTRACT :** This study was conducted to investigate the effect of aflatoxin $B_1$ ($AFB_1$) alone or mixed function oxidase (MFO)-activated $AFB_1$ on various functions of mule duck peritoneal macrophages. Duck peritoneal macrophages were incubated with $AFB_1$ 0, 5, 10, 20, 50 and 100 μg/ml for 12 h. The cell viability significantly declined as the concentration of $AFB_1$ increased and more obviously detrimental effects was noticed in MFO-metabolized $AFB_1$ treatments. Either in opsonized or unopsonized *Candida albicans*, phagocytotic ability of macrophages was decreased with the elevation of the concentration of $AFB_1$. Significantly higher levels of macrophages were damaged in MFO-metabolized $AFB_1$ than $AFB_1$ alone in concentrations above 20 μg/ml. The cytotoxicity activity was in the range of 41 to 33% after exposure to $AFB_1$ 5 to 100 μg/ml, and a significant higher TNF-like substance secretion by lipopolysaccharide (LPS) stimulation was obtained. When LPS was present in the medium, the percentage of cytotoxicity was higher than all treatments of $AFB_1$ both with and without MFO-activation in the absence of LPS. The results suggest that MFO-metabolized $AFB_1$ can alter cell viability and morphology of duck macrophages more than $AFB_1$ administered alone. Both with and without MFO-activation, $AFB_1$ has detrimental effects on phagocytotic ability and TNF-like substance secretion, increasing with level of $AFB_1$. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 3 : 438-444)***

**Key Words :** Aflatoxin $B_1$, Macrophage, Immune Function, Mule Duck

## INTRODUCTION

The high temperature and high humidity in Taiwan provide an environment for the growth of mold in feeds. Aflatoxin (AF) is a secondary metabolite produced in feedstuffs by *Aspergillus flavus* and *Aspergillus parasiticus*. Therefore, the possibility of feed contamination with AF has attracted our attention. The first incidence of aflatoxicosis occurred in turkey which was called turkey "X" disease resulting in the death of over 100 thousand turkeys (Blount, 1961). Although acute intoxication is less likely to occur in current days, a detrimental effect on economic loss due to low dosage of AF contamination should not be neglected (Shen et al., 1988). Once aflatoxin $B_1$ ($AFB_1$) is uptaken by the animals, it is metabolized to its active form in the liver by mixed function oxidase system (MFO) (Swenson et al., 1974). $AFB_1$ itself and its active metabolites may cause toxicological effects, such as mutagenicity, carcinogenicity (Wogan and Newberne, 1966), tetratogenicity and immunosupression (Smith and Moss, 1985). Kadian et al. (1988) demonstrated that specific and non-specific immunosuppression and cellular immune response were inhibited after AF exposure. Peripheral blood lymphocyte transformation after concanavalin A stimulation in porcine and duck was inhibited by $AFB_1$ exposure (Pang, 1994; Cheng and Pang, 1995). $AFB_1$ exposure reduces clearance ability of colloidal carbon particle in the reticulo-endothelial system of chickens (Michael et al., 1973).

Chemotatic and phagocytic activity of blood monocytes are impaired in rabbits fed diets containing AF (Richard and Thurston, 1975) and in heterophils of chickens (Chang and Hamilton, 1979). Percentage of viable cells and functions of turkey macrophages are decreased during aflatoxicosis (Neldon-Ortiz and Qureshi, 1991) and hence implies a decline in disease resistance.

Monocyte-macrophage is a lineage of mononuclear phagocytic system and plays an important role in immuno-defence against pathogenic agents (Skamene and Gros, 1983). There are many functions performed by chicken macrophages, such as phagocytosis of exogenous particles and destruction of abnormal and tumor cells (Qureshi et al., 1986; Qureshi and Miller, 1991). Furthermore, macrophage also can secrete prostaglandin and cytokines to modulate activity of lymphocytes and other macrophages (Kimball, 1990).

The purpose of this experiment was to investigate the effects of $AFB_1$ alone or $AFB_1$ after metabolism by mixed function oxidase from duck liver microsomes on the functions of duck peritoneal macrophages *in vitro* and to elucidate the role in which MFO plays in duck peritoneal macrophages function profile.

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## MATERIALS AND METHODS

### Preparation of duck peritoneal macrophages

In this study, sephadex-elicited peritoneal exudative cells (PEC) were used as the source of duck peritoneal macrophages. The procedure for the preparation of PEC was as previously described by Trembicki et al. (1984). Briefly, a 3% swollen sephadex G-50 suspension in sterile saline (0.75%) was injected intraperitoneally into each duck at 1 ml per 100 g body weight. Approximately 42 h after injection, the ducks were sacrificed by cervical dislocation and PEC were collected by flushing out the peritoneal exudates which were then centrifuged at 285×g for 15 min at 4°C. The resulting pellets of PEC were resuspended in 2 ml of complete medium consisting of RPMI-1640 (Gibco laboratories, Grand Island, NY) supplemented with 10% heat inactivated fetal bovine serum (FBS) (Gibco laboratories, Grand Island, NY) and 2 mM L-glutamine, 100 U/ml of penicillin and 50 μg/ml of streptomycin (all purchased from Sigma, St. Louis, MO). The number of viable PEC was determined by trypan blue exclusion on a hemocytometer and the cell concentration was adjusted to $1\times10^6$ viable cells/ml for the following assays. The overall cell viability was greater than 95% and more than 90% of the cells were duck peritoneal macrophages. For cell preservation, the cell concentration was adjusted to $2\times10^7$/ml in a precooled medium containing 60% RPMI-1640, 20% FBS and 20% dimethylsulfoxide (DMSO; Merck, Darmstadt, Germany) and kept in liquid nitrogen until assay, and the final concentration of DMSO in the incubation media was less than 0.01%.

### Preparation of aflatoxin $B_1$ ($AFB_1$) and mixed function oxidase (MFO)

$AFB_1$ used in this study was obtained from pure crystal $AFB_1$ (Sigma, St. Louis, USA). In preparation of the reagent for assay, $AFB_1$ was first dissolved in DMSO to a concentration of 1 mg/ml as a stock solution. The $AFB_1$ stock solution was then covered with aluminum foil and stored in -20°C for later use.

For $AFB_1$ metabolic activation, microsomes prepared from mule duck were used as the source of MFO. Liver microsomes were prepared from 3 weeks old mule ducks and isolated according to the procedure described by Cook and Hodgson (1983). The protein concentration of microsome preparation was determined by the method described by Lowry et al. (1951) and bovine serum albumin was used as reference protein. The final concentration of 5 mg/ml of microsome protein was used in this study.

### Mixed function oxidase activation

The experiment was divided into two treatments. One was $AFB_1$ treated without MFO-activation and the other was $AFB_1$ treated with MFO-activation. In $AFB_1$ with MFO-metabolized activation before evaluation of the cellular immune functions, duck peritoneal macrophages were cocultured with $AFB_1$ under the effect of MFO (5 mg/ml) and NADPH (0.25 mM) for 12 h. In $AFB_1$ without MFO-metabolized activation, the same volume of 0.2 M phosphate buffer solution was used as a vehicle control instead of microsome. There were no effects of the microsome and NADPH alone on duck peritoneal macrophages viability in our study.

### Evaluation of cytotoxic effect

In the evaluation of cytotoxic effect on duck peritoneal macrophages by $AFB_1$, the concentration for each treatment was 0, 5, 10, 20, 50 or 100 μg $AFB_1$/ml, respectively. During the assay, 50 μl duck peritoneal macrophages ($1\times10^6$ cell/ml) and equal volume of final concentration of $AFB_1$ were added to 96 well microplate and incubated in a humidity incubator at 41°C, 5% $CO_2$ for 12 h. There were 6 replicates for each treatment. When time was up, 10 μl of 5 mg/ml MTT [3-(5,5-dimethylthiazole-z-yl)-2,5-diphenyl tetrazolium] was added per well and incubation was continued for 4 h more, 100 μl acid isopropanol (containing 0.04 N HCl) was added, shaken to dissolve the blue crystal and then OD was measured at 540 nm with ELISA reader.

Lysis of duck peritoneal macrophages with 0.01% Triton X-100 provided a measurement that was considered as 100% lysis. Percentage of viability due to $AFB_1$ exposure was determined by the following formula:

Percentage of viability=[absorbance (duck peritoneal macrophages+medium)-absorbance (duck peritoneal macrophages+sample)÷absorbance (duck peritoneal macrophages +medium)-absorbance (duck peritoneal macrophages+ Triton X-100)]×100.

### Determination of phagocytosis

Phagocytosis was performed by duck peritoneal macrophages on *Candida albican* (ATCC14053) (Culture Collection and Research Center, Food Industry Research and Development Institute, Taiwan, ROC). In this assay, *Candida albican* was incubated for 18-24 h and washed, resuspended with 1 ml heat inactivated duck normal serum, opsonized for 30 min or unopsonized, and then washed twice with phosphate buffer solution, and finally adjusted to $1.0\times10^7$ cell/ml with complete medium.

In order to restore their activity, duck peritoneal macrophages, $1\times10^6$/ml, were cultured for 6 h in a 12 well plate containing a coverslip; the medium was then discarded, fresh medium with various amounts of $AFB_1$ were added, and incubation was continued for a further 12 h. Opsonized or unopsonized *Candida albican* ($1.0\times10^7$ cell/ml) was then added on coverslip to make duck peritoneal macrophages

and yeast ratio at 1:10. After 90 min incubation, the cultures were fixed in methanol and stained with May-Grunwald-Giemsa stain. The percentage of approximately 200 macrophages that had adhered with *Candida albican* was determined by counting each for four coverslips at 1000× magnification in two separate experiments.

**Determination of morphological alteration**

This determination of morphological alteration followed the method of Qureshi and Hagler (1992). The duck peritoneal macrophages after treatment with or without MFO-activation were fixed and stained with May-Grunwald-Giemsa stain. Two hundred macrophages were examined under microscope. Cells showing pseudopodia shrinking, cellular limits losses and disintegration of nucleus were examined at 1000× magnification and scored from each of the three coverslips from two different 12 well plate in each experiment.

**Assay of tumor necrosis factor-like (TNF-like) substance**

The duck peritoneal macrophages ($1.0\times10^6$ cell/ml) after $AFB_1$ exposure, were treated with or without 10 μg LPS/ml and the cells were incubated for 18 h more. When time was up, the sample was centrifuged (800×g) and supernatant was collected for bioassay of TNF-like substance. The method used for assaying TNF-like substance followed the procedure of Qureshi and Hagler (1992) using PK-15 cell line as target cell (Pang et al., 1997). One hundred μl/well of $2.5\times10^4$/ml PK-15 cells in minimal essential medium (MEM) (Gibco laboratories, Grand Island, NY) supplemented with 10% FBS, 2 mM L-glutamine, 100 units/ml of penicillin and 100 μg/ml of streptomycin (MEM-C) were seeded onto flat-bottomed, 96 well plates and cultured for 18 h at 37°C in 5% $CO_2$. After removing the medium, 80 μl/well of MEM-C were added, followed by a 5 fold serial dilution made directly on the plate. All diluted samples were tested in quadruplicate. Series of wells containing PK-15 cells received 80 μl of MEM-C or distilled water to served as the background or positive control, respectively. Following the addition of 80 μl/well of actinomycin D (8 μg/ml), the plates were incubated for another 18 h at 37°C in 5% $CO_2$. The supernatants were discarded and the wells were washed with 200 μl of PBS twice. Twenty microliters of MTT in PBS (5 mg/ml) and 80 μl of MEM-C were then added. Following another 4 h of incubation, the supernatants were removed and 200 μl/well DMSO were added to dissolve the dark blue formazan crystals. The plates were read on an ELISA reader (Bio-Tek, Winooski, VT) using a test wavelength of 550 nm and a reference wavelength of 630 nm. Percent cytotoxicity (TNF-like substance) was determined as [(ODc-ODs)/ODc]×100, where ODc=OD of 100% viable cell control-OD of background control; ODs=OD of test sample-OD of background control. Data were expressed as percentage of control.

**Statistical analysis**

All the data were analyzed statistically according to the General Linear Model procedure of SAS (SAS Institute, 1989). The percentage data were subjected to arc sin transformation prior to analysis of variance and difference among treatments by using Duncan's multiple range test. Student's t-test was used to compare the significant difference of $AFB_1$ without MFO-activation and with MFO-activation and significant values were represented by an asterisk (* $p<0.05$).

## RESULTS

**Cytotoxic effects of $AFB_1$**

The cytotoxic effect of $AFB_1$ on duck peritoneal macrophages is shown in table 1. The percentage of viable cells of macrophages declined with the elevation of concentrations of aflatoxin. There was only 67.1±4.9% survival rate after macrophages which were exposed to $AFB_1$ 100 μg/ml. However, about 20% less viability was noticed after duck peritoneal macrophages were exposed to MFO-activated $AFB_1$ at 100 μg/ml. Significant differences ($p<0.05$) were found between groups with and without MFO-activation for the treatments of $AFB_1$ at 5 to 100 μg/ml.

**Table 1.** Cytotoxic effect of aflatoxin $B_1$ on peritoneal macrophages from mule duck[(1)]

| Concentration of $AFB_1$ (μg/ml) | Viability (%) Without MFO-activation | With MFO-activation | Significance |
|---|---|---|---|
| 0 | 100±0.0[a] | 100±0.0[a] | NS |
| 5 | 94.9±3.3[b] | 86.4±2.7[b] | * |
| 10 | 95.5±1.4[b] | 86.8±2.9[b] | * |
| 20 | 84.2±3.2[c] | 76.2±2.0[d] | * |
| 50 | 84.6±1.8[c] | 71.3±2.0[d] | * |
| 100 | 67.1±4.9[d] | 47.8±3.1[e] | * |

[(1)] Duck peritoneal macrophages used were $1\times10^6$ cell/ml.

MFO: mixed function oxidase. Data are means±SD of 2 different experiments and six replicates were measured for each treatment.

* The difference between treatments without MFO-activation and with MFO-activation at the particular $AFB_1$ concentration is statistically significant ($p<0.05$).

NS: no significant difference in the same row.

[a-e] Values in the same column without common superscripts differ significantly ($p<0.05$).

**Measurement of phagocytosis**

The results of phagocytosis measurement are summarized in table 2. Percentages of phagocytic ability of macrophages on *Candida albican* declined with the elevation of aflatoxin concentration, in both MFO-activation AFB$_1$ and AFB$_1$ alone. The phagocytic ability was significantly lower than the control group after exposure to 10 μg AFB$_1$ (table 2). Similar results were obtained in unopsonized *Candida albican* treatment (table 3). MFO treatment had no effect on the percentage of phagocytic ability of peritoneal macrophages on both opsonized and unopsonized *Candida albican*.

**Table 2.** Effect of aflatoxin B$_1$ exposure on the potential of phagocytosis on opsonized *Candida albicans* by duck peritoneal macrophages(1)

| Concentration of AFB$_1$ (μg/ml) | Phagocytosis (%) | | Significance |
|---|---|---|---|
| | Without MFO-activation | With MFO-activation | |
| 0 | 62.3±1.3$^a$ | 62.3±1.4$^a$ | NS |
| 5 | 60.1±3.0$^a$ | 61.8±1.3$^a$ | NS |
| 10 | 56.4±1.6$^b$ | 56.3±2.6$^b$ | NS |
| 20 | 55.3±2.0$^b$ | 53.9±3.3$^b$ | NS |
| 50 | 36.9±1.1$^c$ | 35.3±1.7$^c$ | NS |
| 100 | 35.6±1.7$^c$ | 33.2±2.3$^c$ | NS |

(1) Duck peritoneal macrophages used were 1×10$^6$ cell/ml.

MFO: mixed function oxidase. Data are means±SD of 2 different experiments and six replicates were measured for each treatment.

NS: no significant difference in the same row.

a-c Values in the same column without common superscripts differ significantly ($p<0.05$).

**Table 3.** Effect of aflatoxin B$_1$ exposure on the potential of phagocytosis on unopsonized *Candida albicans* by duck peritoneal macrophages(1)

| Concentration of AFB$_1$ (μg/ml) | Phagocytosis (%) | | Significance |
|---|---|---|---|
| | Without MFO-activation | With MFO-activation | |
| 0 | 62.6±1.6$^a$ | 62.9±1.2$^a$ | NS |
| 5 | 59.5±0.9$^b$ | 60.8±1.1$^{ab}$ | NS |
| 10 | 56.4±1.9$^c$ | 57.8±2.0$^{bc}$ | NS |
| 20 | 56.5±0.8$^c$ | 56.6±1.3$^c$ | NS |
| 50 | 37.9±1.5$^d$ | 34.8±1.6$^d$ | NS |
| 100 | 35.8±1.0$^d$ | 33.7±3.8$^d$ | NS |

(1) Duck peritoneal macrophages used were 1×10$^6$ cell/ml.

MFO: mixed function oxidase. Data are means±SD of 2 different experiments and six replicates were measured for each treatment.

NS: no significant difference in the same row.

a-d Values in the same column without common superscripts differ significantly ($p<0.05$).

**Morphological alterations**

Table 4 presents morphological alterations in duck peritoneal macrophages after exposure to aflatoxin B$_1$ for 12 h. A horse huff-like cytoplasma, and irregular form of nucleus with ovoid and circle type predominantly appeared on normal peritoneal macrophages from ducks after 12 h incubation. Cytoplasma shrinking, cellular limits losses and disintegration of cells were seen in the damaged macrophages. Granulation and more eosinophilic characteristics were also noticed under light microscope. The amounts of damaged macrophages were linearly increased as the concentration of aflatoxin increased in both with and without MFO-activation treatments. Significant differences ($p<0.05$) were found between MFO metabolized AFB$_1$ and AFB$_1$ alone upon dosages higher than 20 μg/ml.

**Assay of TNF-like substance**

In this assay, lipopolysaccharide (LPS) was applied to elicit the secretion of tumor necrosis factor-like (TNF-like) substance from duck peritoneal macrophages. Changes of TNF-like substance secretion by duck peritoneal macrophages after exposure to aflatoxin B$_1$ or LPS are shown in table 5. The tumoricidal activity was in the range of 41 to 33% after exposure to AFB$_1$ 5 to 100 μg/ml. However, there was a significantly higher TNF-like substance

**Table 4.** Morphological alterations of duck peritoneal macrophages after exposure to aflatoxin B$_1$

| Concentration of AFB$_1$ (μg/ml) | Damaged macrophages(2) (%) | | Significance |
|---|---|---|---|
| | Without MFO-activation | With MFO-activation | |
| 0 | 3.1±0.4$^d$ | 3.4±0.2$^e$ | NS |
| 5 | 3.4±0.2$^d$ | 3.2±0.2$^e$ | NS |
| 10 | 6.6±0.3$^c$ | 7.4±0.9$^d$ | NS |
| 20 | 7.0±1.0$^c$ | 16.7±1.0$^c$ | NS |
| 50 | 12.2±0.9$^b$ | 22.8±2.2$^b$ | NS |
| 100 | 24.7±2.8$^a$ | 34.8±3.0$^a$ | NS |

(1) Duck peritoneal macrophages used were 1×10$^6$ cell/ml.

(2) Cells showed pseudopodia shrinking, cellular limits losses and disintegration at 1000× magnification and were scored from each of the three coverslips from two different 12 well plate in each of two experiments.

MFO: mixed function oxidase. Data are means±SD.

* The difference between AFB$_1$ without MFO-activation and AFB$_1$ with MFO-activation treatments at the particular AFB$_1$ concentration is statistically significant ($p<0.05$).

NS: no significant difference in the same row.

a-e Values in the same column without common superscripts differ significantly ($p<0.05$).

**Table 5.** Changes of tumor necrosis factor-like (TNF-like) substance secretion by duck peritoneal macrophages after exposure to aflatoxin $B_1$ or lipopolysaccharide[(1)]

| Treatment | | Cytotoxicity (%) | | Significance |
|---|---|---|---|---|
| | | Without MFO-activation | With MFO-activation | |
| LPS (10 μg/ml) | $AFB_1$ (μg/ml) | | | |
| - | 0 | - | - | - |
| - | 5 | 41.4±4.8[c] | 40.8±3.3[c] | NS |
| - | 10 | 34.9±3.6[d] | 37.0±1.7[d] | NS |
| - | 20 | 35.9±2.6[d] | 35.4±1.8[d] | NS |
| - | 50 | 34.6±2.5[d] | 36.1±2.0[d] | NS |
| - | 100 | 33.2±3.0[d] | 34.6±2.4[d] | NS |
| 10 | 0 | 62.7±0.8[a] | 63.1±2.0[a] | NS |
| 10 | 5 | 53.1±2.4[b] | 62.4±2.5[a] | * |
| 10 | 10 | 54.0±2.8[b] | 55.6±2.6[b] | NS |
| 10 | 20 | 53.5±1.9[b] | 55.0±2.7[b] | NS |
| 10 | 50 | 53.0±2.6[b] | 55.4±1.8[b] | NS |
| 10 | 100 | 52.2±1.9[b] | 53.9±1.5[b] | NS |

(1) Duck peritoneal macrophages used were $1\times10^6$ cell/ml.

MFO: mixed function oxidase. Data are means±SD of 2 different experiments and six replicates were measured for each treatment.

* The difference between $AFB_1$ without MFO-activation and $AFB_1$ with MFO-activation treatments at the particular $AFB_1$ concentration is statistically significant ($p<0.05$).

NS: no significant difference in the same row.

[a-d] Values in the same column without common superscripts differ significantly ($p<0.05$).

secretion in the lowest dosage of $AFB_1$ than the other higher dosage groups, and a significantly higher TNF-like substance secretion by LPS stimulation was obtained. When LPS was in the medium, the percentage of cytotoxicity was higher than all treatments of $AFB_1$ both with and without MFO-activation in the absence of LPS, and cytotoxicity was slightly decreased as $AFB_1$ levels increased. There was a significant difference between MFO-free and MFO-activation only on treatment with 5 μg/ml of $AFB_1$.

## DISCUSSION

This study showed that the cell viability of duck peritoneal macrophages declined after exposure to $AFB_1$ and the cytotoxic effect of $AFB_1$ on duck peritoneal macrophages was significantly increased as $AFB_1$ was activated by MFO. The shift in the cytotoxic concentration-response relationship upon the MFO-activation was small. This suggests one of three possibilities: (1) $AFB_1$ bioactivation may not be important in damaging the macrophages; (2) duck peritoneal macrophages may activate $AFB_1$ by themselves, and the bioactivating system may only provide incremental metabolism; or (3) the bioactivating system may not be very effective in activating $AFB_1$. In mammals, it has been shown that $AFB_1$ can be activated by cytochrome P450 of MFO in the liver with the existence of oxygen molecule and NADPH (Swenson et al., 1974). It has been also shown that cytochrome P450 is not existing in sephadex elicited chicken peritoneal macrophages (Lorr et al., 1990). However, Karenlampi (1987) indicated that $AFB_1$ could be metabolized by MFO, and an $AFB_1$ 2,3-oxide was formed and further bound to $N^7$-guanine of DNA, which might result in higher cytotoxicity.

There was a significant reduction in phagocytosis as the cells were treated with $AFB_1$ by MFO activation (tables 2 and 3). The chemotaxis and phagocytosis of monocytes and peritoneal macrophages in chicken and turkey are disturbed by $AFB_1$ exposure (Chang and Hamilton, 1979; Neldon-Ortiz and Qureshi, 1991). The possibility of phagocytic depression observed in this study may be related to one or more of the followings reasons as indicated by Qureshi and Hagler (1992) who proposed: (1) inhibition of DNA, RNA and protein synthesis in macrophages after mycotoxin exposure; (2) alterations in metabolic processes, principally glycolysis essential for phagocytosis; or (3) an alteration in the macrophage membrane. These functional alterations may affect RNA function on actin and myosin formation that are essential for chemotaxis and phagocytosis (Aderem and Underhill, 1999). This phenomenon was also demonstrated in alveolar macrophages of pig (Pang, 1994). It is interesting that there was no significant difference between treatments with or without MFO activation, showing that the effect of MFO activated $AFB_1$ on phagocytosis has no additive detrimental effect. This finding is different from the previous report in turkey by Neldon-Ortiz and Qureshi (1991) who revealed the addition of MFO-activated $AFB_1$ resulted in a significant reduction in phagocytic potential. This variation may be caused by the species differences between the two experiments.

The morphological alterations observed in this study were similar to those occurring in alveolar macrophage surface changes of pig after exposure to $AFB_1$ *in vitro* (Pang, 1994). They demonstrated that the damages included pseudopodia shortage and cell blebbing of granulized membrane. Moreover, similar results were also reported by Neldon-Ortiz and Qureshi (1992) who pointed out that morphological changes in chicken peritoneal macrophages after $AFB_1$ exposure included a decline in adherence ability, blebbing formation on cellular surface, and nuclear disintegration.

Macrophages may secrete TNF-like substance following activation by lymphokines and LPS (Hamilton and Adams, 1987). The chicken macropahge cell line MQ-NCSU can endogenously produce low levels of TNF-like substance

and the level of secretion was increased after LPS treatment (Qureshi and Hagler, 1992). In the present study, the data showed that $AFB_1$ treatment suppressed the secretion of TNF-like substance which was in agreement with the previous report (Moon et al., 1999) that cytokine levels in macrophages were depressed by $AFB_1$ treatment. The mechanism by which $AFB_1$ suppresses the production of TNF-like substance is not yet clear. However, there are several possible theories in the suppressive effect for the production of these secretory molecules in the $AFB_1$-exposed macrophages. One is that $AFB_1$ may interfere with the LPS binding to LPS-binding protein on CD14 (Wright et al., 1990), and the other possible mechanism is that $AFB_1$ may down-regulate the expression of CD14, since the expression of CD14 in macrophages stimulated by LPS has been suggested to play a role in the induction of cytokines (Takai et al., 1997).

## CONCLUSION

In this study we demonstrated the impairment of cell viability, morphological changes and decline in phagocytosis in duck peritoneal macrophages after exposure to $AFB_1$ with or without MFO activation. The MFO-activated $AFB_1$ had more obviously detrimental effects on the cell viability and morphology alterations on duck peritoneal macrophages than $AFB_1$ without MFO-activation. High dosage of $AFB_1$ had a negative effect on the secretion of TNF-like substance in duck peritoneal macrophages.

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