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From the National Veterinary Institute, Department of Pathology, Veterinary College, Swedish University of Agricultural Sciences, Uppsala, and Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden.

Ochratoxin A as a Suppressor of Mitogen-Induced Blastogenesis of Porcine Blood Lymphocytes

By *Torbjörn Holmberg, Ann Thuvander* and *Karl Hult*

Holmberg, T., A. Thuvander and K. Hult: Ochratoxin A as a suppressor of mitogen-induced blastogenesis of porcine blood lymphocytes. Acta vet. scand. 1988, 29, 219–223. – The in vitro effect of ochratoxin A on pig lymphocytes stimulated by the mitogen Concanavalin A was studied by measuring the rates of ^{3}H-thymidine incorporation in DNA.

Ochratoxin A inhibited the mitogenic response to Concanavalin A in a dose dependent way. An almost total inhibition was obtained with ≥ 1 mg ochratoxin A/l, approximately 60 % inhibition was produced by 0.5 mg ochratoxin A/l and approximately 10 % inhibition by 0.06 mg ochratoxin A/l.

The immunosuppressive effect of ochratoxin A was not altered much by different contents of bovine serum albumin, 0.1 or 0.3 %, in the cell culture medium.

immunosuppression; lymphoblastogenesis; pigs.

Introduction

Ochratoxin A, a fungal metabolite produced by several species of Aspergillus and Penicillium, has a well documented nephrotoxic effect (*Krogh* 1978) causing spontaneous nephropathy in pigs and chickens. Ochratoxin A also causes immunosuppression (*Szczech et al.* 1973a, b, *Prior & Sisodia* 1982, *Dwivedi & Burns* 1984). Even single parenteral administration of a low dose of ochratoxin A to mice reduced their antibody response to sheep erythrocytes (*Haubeck et al.* 1981, *Creppy et al.* 1983). *Haubeck et al.* (1981) suggest that ochratoxin A at levels found in blood of slaughter pigs (for a review see *Hult & Fuchs,* 1986) have a negative effect on the pig's immune system.

Inhibition of the lymphocyte blastogenesis is a valid in vitro model for determining the effect of environmental contaminants on immunocompetent cells (*Kristensen et al.* 1982) and has been used to study the effect of mycotoxins on blood lymphocytes (*Paul et al.* 1977, *Cooray* 1984, *Forsell & Pestka* 1985). In the present study we used the lymphocyte stimulation test to elucidate the immunosuppressive effect of ochratoxin A on lymphocytes obtained from young growing pigs.

Materials and methods

Animals

From a litter of 3 months old special pathogen free (SPF) pigs, clinically healthy and housed under strict isolation conditions, 6 animals were randomly selected and used as blood donors.

Ochratoxin A

A strain of Penicillium nordicum was cultivated on barley for 2 weeks at 25°C. Crystalline ochratoxin A was prepared from the culture according to the method for extraction and purification described by *Fuchs et al.* (1984).

Lymphocyte stimulation test

Peripheral blood lymphocytes were isolated from heparinized venous blood. Blood samples were diluted with an equal volume of RPMI 1640 cell culture medium, pH 7.4, carefully layered on a caushion of Ficoll-Paque (d = 1.077 g/ml; Pharmacia, Uppsala, Sweden) and centrifuged for 25 min at 400 g. The cells from the interface were collected and washed 3 times in phosphate buffer saline (0.01 mol/l sodium phosphate, 0.85 % sodium chloride, pH 7.2). The cell pellet was resuspended in RPMI 1640 supplemented with 20 mmol/l HEPES, 2 mmol/l glutamin, 200 IU/ml penicillin and 200 µg/ml streptomycin.

The cells were cultured in plastic tubes at 1×10^6 cells per tube in a volume of 1 ml. The mitogen Concanavalin A (Con A) was added at a final concentration of 2.5 µg/ml to all tubes except for the negative control cultures. Ochratoxin A, diluted in RPMI 1640 medium, was added at concentrations between 0.06–4 mg/ml to the cultures with Con A.

The various cell cultures were incubated for 3 days at 37°C, whereupon 1 µCi ^{3}H-thymidine was added. After another 24 h of incubation, the cells were harvested with a semiautomatic cell culture harvester on glass microfiber filters and were dried and transferred to vials containing scintillation liquid (Lipo Luma/3 M bv, The Netherlands) The ^{3}H activity of each culture was measured in a liquid scintillation counter (Minaxi, Tricarb 4000 β-counter, United Technologies, Packard Instruments). All results were expressed as the average counts per minute (cpm) of triplicate cultures. The stimulation index (SI) was calculated according to the formula:

$$SI = \frac{\text{Mean cpm of the Con A stimulated cultures}}{\text{Mean cpm of the negative control cultures}}$$

The percentage of inhibition (IH %) of DNA synthesis exerted by ochratoxin A was calculated according to the formula:

$$IH\ \% = 100 - \frac{\text{mean cpm of Con A stimulated cultures with ochratoxin A} \times 100}{\text{mean cpm of Con A stimulated cultures without ochratoxin A}}$$

Medium supplement

The assay was performed with 2 different concentrations of bovine serum albumin (BSA) in the medium. Therefore, RPMI 1640 cell culture medium was supplemented with 10 % fetal calf serum (FCS) or 2 % Ultroser G (LKB, Solna, Sweden). Analysis of the serum albumin content in FCS and Ultroser G was performed at the Department of Clinical Chemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Results

The blood lymphocytes responded to Con A with SI values ranging from 18 to 101 in cultures supplemented with FCS (extremes from animals not shown in Table 1) and with SI from 69 to 134 in cultures supple-

Tabel 1. The effect of ochratoxin A on mitogen (Con A) stimulated porcine blood lymphocytes cultured in medium supplemented with 10 % fetal calf serum (FCS; 0.3 % BSA in the medium) or with 2 % Ultroser G (0.1 % BSA in the medium). The results represent 3 different animals and the standard deviation (SD) of triplicate cultures from each animal. ND = not done.

Medium supplement	Ochratoxin A mg/l	Animal no. 10		Animal no. 12		Animal no. 16	
		^{3}H-thymidine cpm x 1000 ± SD	SI	^{3}H-thymidine cpm x 1000 ± SD	SI	^{3}H-thymidine cpm x 1000 ± SD	SI
FCS	0	7 ± 2	1	4 ± 0.4	1	4 ± 3	1
FCS + Con A	0	341 ± 21	49	152 ± 10	38	181 ± 24	45
	0.06	312 ± 23	45	ND	–	ND	–
	0.12	311 ± 41	44	ND	–	ND	–
	0.25	295 ± 23	42	118 ± 23	29	103 ± 13	26
	0.5	203 ± 32	29	53 ± 4	13	66 ± 27	16
	1	46 ± 18	7	12 ± 2	3	5 ± 0.7	1
	2	2 ± 1	0.3	1 ± 0.5	0.2	1 ± 0.0	0.2
	4	ND	–	0.7 ± 0.3	0.2	0.4 ± 0.1	0.1
Ultroser G	0	3 ± 0.9	1	2 ± 0.3	1	1 ± 0.6	1
Ultroser G + Con A	0	208 ± 15	69	227 ± 12	113	134 ± 12	134
	0.06	184 ± 25	61	ND	–	ND	–
	0.12	159 ± 9	53	ND	–	ND	–
	0.25	141 ± 14	47	151 ± 31	75	102 ± 13	102
	0.5	103 ± 5	34	62 ± 4	31	42 ± 6	42
	1	2 ± 2	0.7	2 ± 1	1	0.9 ± 0.4	0.9
	2	0.4 ± 0.0	0.1	0.5 ± 0.1	0.2	0.4 ± 0.2	0.4
	4	ND	–	0.4 ± 0.1	0.2	1 ± 0.2	1

mented with Ultroser G. The addition of ochratoxin A resulted in an inhibition of the mitogen induced lymphoblastogenesis as measured by ^{3}H-thymidine incorporation in the cells. The inhibition was dose dependent compared with stimulated cultures without ochratoxin A (Fig. 1). An almost total inhibition (95–100 %) was observed with concentrations of ochratoxin A $\geq$ 1 mg/l. Approximately 60 % and 10 % inhibition of the lymphocyte response was noticed with 0.5 and 0.06 mg ochratoxin A/1, respectively.

The serum albumin content in FCS and Ultroser G was 30 and 57 g/l respectively. The addition of 10 % FCS or 2 % Ultroser G to the culture medium gave final BSA-concentrations of 0.3 % or 0.1 % respectively. No marked difference in inhibition of lymphoblastogenesis caused by ochratoxin A was seen between cultures supplemented with FCS or Ultroser G (Table 1). Only a tendency to higher immunosuppression by ochratoxin A was noticed for cultures supplemented with less BSA (Ultroser G).

Discussion

Ochratoxin A is known to act in a non-selective immunosuppressive manner causing both reduction in the number of antibody forming cells in lymfoid organs (*Prior & Sisodia* 1982) and reduction of total immunoglobulin levels (*Creppy et al.* 1983,

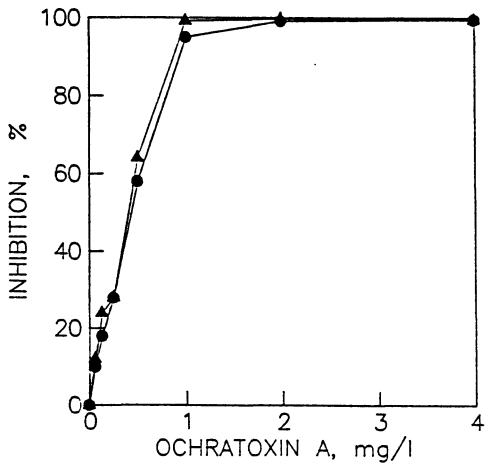

Figure 1. The inhibitory effect of different doses of ochratoxin A on the response of lymphocytes obtained from SPF-pigs to Concanavalin A as measured by ^{3}H-thymidine incorporation.
(●) % inhibition in cultures supplemented with fetal calf serum (means from 6 animals).
(▲) % inhibition in cultures supplemented with Ultroser G (means from 3 animals).

Dwivedi & Burns 1984). This mode of action is probably due to the strong inhibition of protein synthesis demonstrated for ochratoxin A (*Röschenthaler et al.* 1984).

In the present experiment the effect of ochratoxin A on in vitro stimulated porcine blood lymphocytes was studied. The mitogen used, Con A, mainly stimulates the thymus derived (T) lymphocytes. Our findings demonstrated the capacity of ochratoxin A to bring about a dose dependent in vitro inhibition of cell mediated immune response in the pig. Using higher concentrations of ochratoxin A similar immunosuppressive effects have been reported for mitogen stimulated lymphocytes from mice (*Prior & Sisodia* 1982) and humans (*Cooray* 1984).

Ochratoxin A binds to BSA (*Chu* 1971). We therefore compared media supplemented with either 10 % FCS or 2 % Ultroser G, giving different final BSA contents in the media, 0.3 % or 0.1 % respectively. According to *Chu* (1971) this difference in BSA concentration changed the ratio between the free and the bound forms of the toxin so that the concentration of free ochratoxin A in the low BSA medium was twice that in the high BSA medium. The immunosuppressive effect of ochratoxin A was, however, not much affected by the higher BSA-content in the cell culture medium. Therefore, our study indicates that immunosuppression caused by ochratoxin A is not only a function of the amount of free toxin present for the cells. This is in accordance with other studies (*Haubeck et al.* 1981, *Creppy et al.* 1983) where potent immunosuppressive effects of low doses of ochratoxin A in mice have been demonstrated despite the possibility for ochratoxin A to bind to serum albumin.

Our experiment was carried out using a limited number of young pigs. Nevertheless the result is interesting because it indicates that the pig's immune system is sensitive even to doses of ochratoxin A considerably lower than 1 mg/l. From the dose-response graph (Fig. 1) it can be seen that a 10 % inhibition of the lymphocyte response occurs at approximately 0.06 mg ochratoxin A/l, a level of ochratoxin A which is naturally found in the blood of slaughter pigs (*Hult et al.* 1980, *Hult et al.* 1984). As a consequence of this fact the long time effects in pigs exposed to ochratoxin A blood levels of 0.06 mg/l or even lower need to be evaluated. Under practical farming conditions a 10 % inhibition of the immune response might have a great relevance to the health of the pigs.

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Sammanfattning

Ochratoxin A som hämmare af mitogeninducerad blastogenes av lymfocyter från grisar.

Effekten in vitro av ochratoxin A på stimulering av grislymfocyter med mitogenet Concanavalin A studerades genom att mäta mängden ^{3}H-thymidin som inkorporerades i lymfocyternas DNA.

Ochratoxin A hämmade mitogeneffekten av Concanavalin A på ett dosrelaterat sätt. En nästan total inhibering erhölls med ≥ 1 mg ochratoxin A/l, ungefär 60 % inhibering åstadkoms med 0,5 mg ochratoxin A/l och ungefär 10 % inhibering med 0,06 mg ochratoxin A/l.

Den immunosuppressiva effekten av ochratoxin A påverkades inte i nämnvärd omfattning av olika mängder av bovint serum albumin, 0,1 eller 0,3 %, i cellodlingsmediet.

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Reprints may be requested from: Torbjörn Holmberg, National Veterinary Institute, P. O. Box 7073, S-750 07 Uppsala, Sweden.