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NATIONAL INSTITUTE OF PUBLIC HEALTH AND THE ENVIRONMENT

RIVM report 388802 017

Immunosuppressive effects of fumonisin B_1 in the *Trichinella spiralis* model

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This investigation has been performed by order and for the account of the Inspectorate for Health Protection, Commodities and Veterinary Public Health, within the framework of project 388802, Natural toxins.

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Abstract

Rationale
Fumonisin B_1 is a mycotoxin produced by *Fusarium moniliforme* and is found mainly in maize. Fumonisin B_1 has been associated with human esophageal cancer, lung edema in pigs and leuko-encephalomalacia in equine species. Adverse effects of this mycotoxin on the immune system can be expected but were currently only reported in chickens and mice. The toxic effects of this mycotoxin in male rats has been studied in a 28-days toxicity study (TIER I) in the RIVM:WU rat (AAP protocol 199600652). The weight of the kidneys of animals in the high dose group were significantly decreased as compared to weights of kidneys of animals in the lower dose and control groups. Pathological effects were observed in kidneys of animals of all dose groups. Dose related single cell necrosis was observed in all dose groups. The activity of γ-GT was significantly increased in animals in the highest dose group. No direct immunotoxic effect was observed, however, there was a trend for an increased concentration of B-cells in animals in the highest dose group.

In a functional assay (*Trichinella spiralis* infection) the effect of low doses of fumonisin B_1 on the immmune system were studied.

Methodology
Male rats (age 3 weeks) received oral (gavage) doses of 0, 0.05, 0.19 or 0.75 mg fumonisin B_1 kg^{-1} body weight (BW) (dissolved in water) daily for 10 weeks. All animals were infected with larvae of the *Trichinella spiralis* on day 28. After 70 days of treatment, the animals were sacrificed and necropsied. Specific antibody titers were determined in the blood. Larvae were counted in the whole carcass and tongue tissue.

Results
The growth of animals of the treatment groups was decreased, not statistically significant, as compared to growth of animals in the control group. All immunogobulin levels were decreased, IgG and IgM statistically significant, in animals of the mid and high dose groups as compared to animals of the control group. IgG was statistically significantly decrease in the low dose groups as compared to animals of the control group. The relative amount of *T. spiralis* larvae in tongue muscle was increased in animals of all treatment groups, statistical significance was observed in the lowest and highest dose group. No differences in inflammatory reaction surrounding the larvae in the tongue muscle were observed. Total count of larvae present in the carcasses was increased in animals in the mid and high dose groups (not statistically significant). It was lowered in the low dose group. Thus, a trend was observed for increased counts of larvae when the animals were exposed to higher levels of fumonisin B_1.

Conclusion
The immune system of rats is functionally affected after chronic exposure to low doses fumonisin B_1, although nephrotoxicity is found at yet lower dosage levels.

Contents

Samenvatting

Rationale
Fumonisine B_1 is een mycotoxine geproduceerd door *Fusarium moniliforme* en wordt vooral gevonden in mais. Fumonisine B_1 veroorzaakt esophagus kanker in de mens, longoedeem in het varken en leuko-encephalomalacie in het paard. Effecten van dit mycotoxine op het immuunsysteem werden waargenomen in de kip en de muis. Het toxische effect van Fumonisine B_1 werd bestudeerd in een 28-daagse toxiciteitsstudie in de RIVM:WU rat. In dat experiment werd het gewicht van de nieren in de hoogste doseringsgroepen significant verlaagd. Pathologische effecten werden gevonden in nieren van alle doseringsgroepen. Voorts werd dosis-gerelateerde celnecrose gevonden in alle doseringsgroepen. Het effect van γ-GT was significant verhoogd in dieren uit de hoogste doseringsgroepen. Geen direct immunotoxisch effect werd waargenomen, hoewel er een trend werd gezien in toegenomen B-cel aantallen in dieren uit de hoogste doseringsgroepen.

Methode
In deze studie werd het effect van lage doseringen Fumonisine B_1 op het immuunsysteem bestudeerd met behulp van een functionele assay, het *Trichinella spiralis* infectiemodel. Mannetjesratten van drie weken oud werden oraal via maagsonde behandeld met 0, 0,05, 0,19 of 0,75 mg Fumonisine B_1 per kg lichaamsgewicht dagelijks gedurende 10 weken. Vervolgens werden alle dieren oraal geïnfecteerd met *Trichinella spiralis* larven. Zeventig dagen na het begin van het experiment werden dieren opgeofferd, antilichamen werden in het bloed gemeten, terwijl *Trichinella spiralis* larven in het karkas en tongweefsel werden gemeten. Het gewicht van de dieren in alle behandelde groepen was wat afgenomen, echter de afname was niet statistisch significant. IgE- en IgG-antilichaamtiters waren statistisch significant afgenomen in de midden en hogere doseringsgroepen, terwijl de IgG-antistoftiter ook significant was afgenomen in de laagste doseringsgroep. Het aantal *Trichinella spiralis* larven in tongweefsel was statistisch significant toegenomen in alle doseringsgroepen. De ontstekingsreactie om larven in tongweefsel waren kwalitatief en kwantitatief niet beinvloed door Fumonisine B_1 blootstelling en in het gehele karkas werden toegenomen aantallen *Trichinella spiralis* larven gezien, doch deze toename was niet significant.

Conclusie
Het immuunsysteem van ratten wordt nadelig beinvloed in zijn functie naar chronische expositie aan lage doseringen van Fumonisine B_1.

1 Introduction

Fumonisin B_1 is a mycotoxin produced by the field fungi *Fusarium moniliforme* and *F. proliferatum* and is found mainly in maize. Due to climate conditions all maize for human consumption is imported in The Netherlands. Exposure of the population in The Netherlands to fumonisin B_1 will probably increase in the future since maize consumption by the population is becoming more and more popular. Nevertheless, total level of exposure of people in The Netherlands to fumonisin B_1 is expected to be low. It is therefore important to know the toxic effects of low levels of fumonisin B_1.

Fumonisin B_1 has been associated with human esophageal cancer, lung edemia in pigs (PPE) and leuko encephalomalacy in equine species (ELEM) (Dutton 1996). The mycotoxin is hepatotoxic and was characterized as a tumor promotor in rat liver (Gelderblom *et al.* 1988, 1991). Severe toxic effects of fumonisin B_1 to the kidneys, such as single cell necrosis, have been found in test animals (Suzuki *et al.* 1995; Voss *et al.* 1995). The toxic dose for renal toxicity is lower than for hepatotoxicity (Riley *et al.* 1994).

Effects on the immune system have been observed in rats (Bondy *et al.* 1995). Weight of thymus was significantly reduced, disseminated thymic necrosis was observed and immunoglobulin M levels were consistently elevated in rats treated intraperitoneally with fumonisin B_1. Changes in the expression of receptors that modulate T-cell-mediated immunity have been observed in mice after ip administration of fumonisin B_1 (Martynova *et al.* 1995). Total Ig and IgG levels were significantly decreased and reduced phagocytic activity by macrophages was observed in chickens (white leghorns) receiving *F. proliferatum* culture material via feed (Qureshi *et al.* 1995).

Kidney and liver were identified as the prime target organs for toxicity of low doses of fumonisin B_1 in a 28-days study. Exposure to low fumonisin B_1 levels induced continuous cell proliferation in the kidney tubules which might eventually result in tumor alterations in the proliferating cells. No clear effects were observed on the immune system. However, when the observed trends in that experiment were compared with the results from other published experiments on the parameters of the immune system, potential immunotoxicity of fumonisin B_1 could not be ruled out (de Nijs et. al., 1999).

The enzyme ceramide synthase that catalyses the sphinganine-to-sphingosine transformation is blocked by fumonisin B_1 (Spiegel and Merrill 1996). The ratio of the free forms of the two sphingolipids could, thus, be regarded as biomarker for fumonisin B_1 exposure. The free sphingolipids can be determined in serum, tissues of liver and kidney and in urine (Riley *et al.* 1994).

The described experiment was carried out to gain knowledge on the functional effects (Tier II) of the low immunotoxic doses of fumonisin B_1.

2 Materials and Methods

2.1 Deviations from the study plan

No serum was preserved for further analysis on sphingolipids. During the first experiment in AAP 199600952, it was shown that changes in the sphingolipid composition was not clear when measured in blood serum. These results were obtained after the proposal AAP 199800065 was prepared.

2.2 Test organisms

Male RIVM:WU rats were ordered from RIVM CDL-APG at weaning age, about 3 weeks old. Four groups of 10 rats were treated with fumonisin B_1 (Table 1) after an acclimatization period of one week. The animals were housed individually in macrolon cages. The identification number of the animals was written on the tail using a marker pen.

Table 1. Treatment schedule.

Group	Animal numbers	Treatment	Dosage mg fumonisin B_1 per kg rat
1	01-10	control	0
2	11-20	low dose fumonisin B_1	0.05
3	21-30	medium dose fumonisin B_1	0.19
4	31-40	high dose fumonisin B_1	0.75

2.3 *T. spiralis*

A suspension of 1000 larvae of *T. spiralis* in 0.2% agar purum were prepared according to SOP PMP008 and PMP009. Administration was carried out via intubation, 0.5 ml 100 g^{-1} BW.

2.4 Husbandry and treatment

All animals were housed in the animal room in building D6D. Air pressure was controlled at 15 ± 5 Pa., temperature was set at 20-24°C and relative humidity at 45-65%. The air pressure, relative humidity and temperature were controlled daily. A note was made in the labjournal in case of deviations of the set limits. The labjournal is filed.

After the acclimatization period, the animals were treated orally (gavage: 0.5 ml 100 g^{-1} BW) for 70 days with daily doses of 0, 0.05, 0.19 or 0.75 mg fumonisin B_1 kg^{-1} BW dissolved in distilled water. Drinking water and food (RHM-GS flour) were provided *ad libitum*.

2.5 Test substances

Fumonisin B_1 was provided by Dr. E. Sydenham, Medical Research Council, Programme on Mycotoxins and Experimental Carcinogenesis, Tygerberg, South Africa.

The fumonisin B_1 was dissolved in distilled water according to RIVM-ARO protocol 1.1997.05. One batch of stock solution was made at the start of the project.

2.6 Chemical analysis of dose preparations

Concentrations of the dilutions made from the fumonisin B_1 stock solution for the animal experiments were controlled just before the solutions were delivered at the animal facilities. The concentrations were controlled again in the left-over of each bottle according to RIVM-ARO protocol 1.1997.05. The results of these experiments are presented in a letter report RIVM-ARO 1.1997.05.

2.7 Observations

Clinical signs and mortality were checked daily. Body weight was recorded three times per week. Water and food intake were not recorded.

2.8 Analysis and pathology

Specific antibodies against *T. spiralis* were determined according to SOP LPI274 (IgA-a-Ts), LPI275 (IgE-a-Ts), LPI021 (IgG-a-Ts) and LPI273 (IgM-a-Ts). Autopsy was performed one day after the last treatment (days 70).

Blood was withdrawn from the animals before exsanguination for analysis of specific antibody titers.

After exsanguination of the animals (abdominal aorta under ether anesthesia) the tongue was fixed in 10% buffered formaldehyde and prepared for analyses (SOP PMP008, SOP PMP009). Inflammory reactions surrounding the larvae in muscles are recorded semiquantitatively. The carcass was prepared for total larvae count (SOP PAT316). Macroscopic histopathology findings are recorded.

3 Results

3.1 Analysis of dose preparations

The results of the analysis of the fumonisin B_1 solutions are described in the RIVM-ARO letterreport 1.1997.05. No deviations in concentration were found in the concentrations of all fumonisin B_1 solutions freshly prepared and after use in animal experiment. The stock solution was stable for at least the 10 week period investigated.

3.2 Observations

3.2.1 Clinical observations

No clinical observation were made during the experiments.

3.2.2 Mortality

No animals died during the course of the experiment.

3.2.3 Body weight (growth)

Table 2 shows the body weight (g) and body weight gain (g/10 weeks). Figure 1 shows the growth curves of the average per group.

Table 2. Body weight (g) and body weight gain in 4 weeks (g) of rats exposed to fumonisin B_1 (Mean ± SD) and infected with *T. spiralis*.

	Group 1 Control	**Group 2** Low dose	**Group 3** Mid dose	**Group 4** High dose	p*
week -1	69±9	71±13	71±12	69±12	0.9625
week 1	98±8	99±23	105±15	98±19	0.7666
week 2	150±10	153±16	154±13	144±17	0.3623
week 3	197±15	196±19	202±15	189±17	0.4025
week 4	243±20	240±18	248±14	231±17	0.2107
week 5	292±28	285±16	294±20	275±17	0.1841
week 6	322±32	312±16	322±21	303±17	0.2010
week 7	349±37	335±15	343±20	328±19	0.2589
week 8	377±41	359±15	369±29	354±21	0.2728
week 9	401±44	381±17	389±30	376±23	0.3113
week 10	415±46	401±15	404±29	396±26	0.5667
week 11	429±47	405±15	416±29	408±27	0.3557
Body weight gain (4 weeks)	331±45	306±21	312±30	310±35	0.3762

*p-value of 1-factor ANOVA

No statistical significant differences in growth rate could be observed during the administration period.

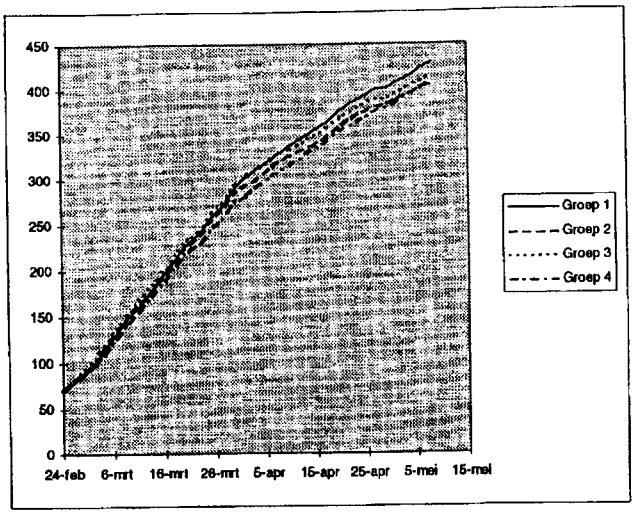

Figure 1. Growth curves of rats exposed to fumonisin B_1 (Mean ± SD) and infected with *T. spiralis*.

3.3 Clinical Laboratory Investigations

3.3.1 Specific antibody titers

Specific antibody titers are expressed as percentage of a standard of pooled control sera.

Table 3. Specific antibody titers of rats exposed to fumonisin B_1 (Mean ± SD) and infected with *T. spiralis*. The number of animals per group is given in brackets if not 10.

	Group 1 Control	**Group 2** Low dose	**Group 3** Mid dose	**Group 4** High dose	p*
IgA-a-Ts	5.3±1.6	5.8±0.9	4.3±1.2	4.7±1.7	0.0930
IgE-a-Ts	8.5±2.1	8.1±1.1	6.7±1.6	7.9±2.6	0.2047
IgG-a-Ts	9.0±1.3	7.7±0.8[a]	7.6±1.0[a]	7.2±1.2[b]	0.0055
IgM-a-Ts	7.5±2.6	7.9±1.07	7.3±1.2	5.5±1.7	0.0318

* p-value of 1-factor ANOVA
[a] $p<0.05$ between control and treatment group
[b] $p<0.01$ between control and treatment group

The ANOVA table showed inhomogenity for the IgM-a-Ts titers. No significant differences were observed between the highest dose group and the control group.

A trend showing decreased specific antibody titers was observed for all antibodies tested. This should be regarded as a biological result of the exposure to fumonisin B_1.

3.3.2 Survival

There were no unscheduled deaths or intercurrent kills of animals

3.3.3 Larvae of *T. spiralis* in carcass

The results of the counts of the larvae in the carcass are presented in Table 4.1 and 4.2. No significant differences were observed between the dose group and the control groups. There is a trend in increased larvae number with increased dose of mycotoxin.

Table 4.1. Larval counts (number of larvae per carcass) of carcasses of rats exposed to fumonisin B_1 (Mean ± SD) and infected with *T. spiralis*. The number of animals per group is given in brackets if not 10.

			p*
Group 1	Control	32040±21448	
Group 2	Low dose	21540±27517	
Group 3	Mid dose	39440±20993	
Group 4	High dose	39170±24228	0.3491

* p-value of 1-factor ANOVA

Table 4.2. Relative larval counts (number of larvae per g) of rats exposed to fumonisin B_1 (Mean ± SD) and infected with *T. spiralis*. The number of animals per group is given in brackets if not 10.

			p*
Group 1	Control	74±51	
Group 2	Low dose	52±67	
Group 3	Mid dose	95±54	
Group 4	High dose	96±63	0.2997

* p-value of 1-factor ANOVA

3.3.4 Larvae of *T. spiralis* in tongue

The results are presented in Tables 5 and 6. Administration of fumonisin B_1 resulted for the highest dose investigated in a statistically significantly increase the amount of larvae of *T. spiralis* in the tongue of rats in this experiment. No differences were observed for inflammatory reaction surrounding the larvae.

Table 5. Larval counts (mean number of muscle larvae per mm square) of tongues of rats exposed to fumonisin B_1 (Mean ± SD) and infected with *T. spiralis*. The number of animals per group is given in brackets if not 10.

			p value*
Group 1	Control	0.478±0.14	
Group 2	Low dose	0.607±0.16 (9)	0.0759 (**0.0379**)
Group 3	Mid dose	0.616±0.22	0.1138 (0.0569)
Group 4	High dose	0.620±0.11	**0.0217 (0.0108)**

* p-value of t-test two tailed. Within brackets one tailed t-test. Bold face statistically significant at $p<0.05$.

Table 6. Effect of fumonisin B_1 treatment on inflammatory response around muscle larvae of *T. spiralis* in the tongue in control and highest dose group (Mean percentage of total number of larvae present ± SD). The number of animals per group is given in brackets if not 10.

Dose	None[a]	Minimal	Moderate	Marked	Total
0	58±5[b]	30±3	9±3	4±2	100±0
0.75	57±8	30±7	9±4	3±2	100±0

[a] Semiquantitative evaluation of inflammatory response around muscle larve. None, no or a few cells present; Minimal, some inflammatory cells present; Moderate, some layers of cells surrounding muscle larve; Marked, several cell layers surrounding muscle larve and/or encapsulated larvae or tissue replaced by inflammation.

[b] Mean and standard deviation in percentage of total number of larvae present. Number of larve counted and evaluated for inflammatory response in two sections of the tongue.

4 Discussion and conclusions (evaluation)

4.1 Discussion

The presented 70-days infection model study on immunotoxicity of fumonisin B_1 in rats, showed that total body weight and rate of increase of body weight of treated animals was lower than the control group, although not statistically significantly. A statistically significant decrease of antibodies G and M, specific to *Trichinella spiralis*, was observed in animals in the mid and high dose groups as compared to the control group. Total counts as well as the relative count of muscle larvae in the carcass were increased in animals in the mid and high dose groups as compared to the control group, although, the increase was not statistically significant, which is primarily due to the large variation. The amount of larvae in the tongue muscle was statistically significantly increased in the highest dose group.

4.2 Conclusions

The presented 70-days immunotoxicity of fumonisin B_1 in rats, with *T. spiralis* as infectious agent, showed an increase in striated muscle larvae in animals treated with high doses (0.19 and 0.75 mg kg^{-1} BW) of fumonisin B_1. Statistically significant more larvae were present in tongue muscle in the highest dose group. It can be concluded that the immune system of rats can be affected after chronic exposure to low doses fumonisin B_1.

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Appendix 1 Organization

General

Title	: Vaststellen van immuunsuppresieve effecten van fumonisine B_1 in het *T. spiralis* model Immunosuppresive effects of fumonisin B_1 in the *T. spiralis* model
On behalf of	: Ir.W.de Koe, Inspectie W&V
Study director	: Dr.H.van Loveren (RIVM/LPI)
Testing facilities	: National Institute of Public Health and The Environment (RIVM), Antonie van Leeuwenhoeklaan 9, Bilthoven, The Netherlands.
Head laboratory	: Dr. J.G. Vos (RIVM/LPI)
Study number	: 199600065

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Histochemistry	: S.G.P.de Waal-Jacobs
Histopathology	: Dr.W.H.de Jong (RIVM/LPI)

Schedule

Delivery of animals and Acclimatization	: 23-02-1998
Allocation	: 02-03-1998
Administration start	: 02-03-1998
T. spiralis infection	: 30-03-1998
Termination	: 11-05-1998

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