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Oxidative Stress Induced by Fumonisin B_1 in Continuous Human and Rodent Neural Cell Cultures

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Fumonisin B_1 (FB_1) is a mycotoxin produced by *Fusarium verticillioides*, which is a common infectant of corn and other cereal grains. Of concern to human health is also a possible airborne exposure to FB_1-producing strains of *F. verticillioides*, which may grow in moisture-damaged buildings. In this study, we have characterized oxidative stress-related parameters induced by FB_1 in three different neural cell lines, human SH-SY5Y neuroblastoma, rat C6 glioblastoma and mouse GT1-7 hypothalamic cells. The cells were exposed to graded doses of FB_1 between 0.1 and 100 μM for 0–144 h after which the production of reactive oxygen species (ROS), lipid peroxidation, intracellular glutathione (GSH) levels and cell viability were measured. FB_1 caused a dose-dependent increase of ROS production in C6 glioblastoma and GT1-7 hypothalamic cells but was without an effect in SH-SY5Y cells. Decreased GSH levels, increased MDA-formation, indicative of lipid peroxidation and necrotic cell death were observed in all cell lines after incubation with FB_1. These findings indicate that FB_1 induces oxidative stress in human, rat and mouse neural cell cultures.

Keywords: Fumonisin B1; Reactive oxygen species; Lipid peroxidation; Glutathione; Cytotoxicity; Neurotoxicity

INTRODUCTION

Fumonisins are secondary metabolites produced by the fungus *Fusarium verticillioides* (= *F. moniliforme*), which commonly contaminates corn across the world.[1] Fumonisin B_1 (FB_1) is the most prevalent among all classes of fumonisins (A, B, C), and it is frequently present at high concentrations in food items contaminated by FB_1-producing *F. verticillioides*. At high doses, FB_1 causes porcine pulmonary oedema[2,3] and leukoencephalomalacia in horses[4,5] and rabbits.[6] It has been suggested that FB_1-induced cardiovasular dysfunction in horses may contribute to the development of leukoencephalomalacia in these animals.[7] Toxic effects of FB_1 on the liver and kidney have been extensively studied in rats and mice.[8] FB_1 induces renal tubular tumours in male rats and hepatic tumours in female mice.[9,10] Consumption of FB_1-contaminated corn has been associated with high frequencies of human oesophageal cancer in certain areas of South Africa and China.[11,12] In addition to contaminated foods and feeds, different species of *Fusarium* are a frequent finding in moisture-damaged buildings.[13,14] Thus, FB_1-producing *F. verticillioides* may grow in mould-contaminated buildings, raising the concern of a possible human exposure to this and other mycotoxins.[15,16]

FB_1 affects cells via the inhibition of ceramide synthase (sphingosine *N*-acyltransferase), a key-enzyme in *de novo* sphingolipid biosynthesis and sphingolipid turnover. This inhibition takes place because of marked similarities between the molecular structures of FB_1 and long-chain (sphingoid) base backbones of sphingolipids.[17,18] The disruption of the normal sphingolipid metabolism leads to elevated levels of free sphingoid bases and sphingoid base metabolites. FB_1-induced inhibition of ceramide synthesis can result in a wide spectrum of changes in lipid metabolism and associated lipid-dependent

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signalling pathways. This appears to be a major contributor to the carcinogenic and other deleterious effects of FB_1.[19] The sphingolipid depletion caused by FB_1 is also thought to reduce folate uptake in mice, humans and cell-lines, and thereby to induce neural tube defects.[20–23]

Several studies have reported mechanisms whereby FB_1 may affect brain and neuronal cells. In rat C6 glioma cells, FB_1 inhibits protein synthesis, causes DNA fragmentation and cell death, and induces lipid peroxidation. Cytotoxic concentrations of FB_1 induce cell cycle arrest in C6 cells, possibly associated with genotoxic events.[24,25] FB_1 also causes DNA damage and induces caspase-3 activity, suggesting a role in a cascade of events leading to apoptosis, in rat astrocytes.[26] Significantly increased sphinganine levels have been observed in the forebrain and brainstem of rats treated with FB_1. The same study[27] also revealed demyelination in the forebrain of rats treated with FB_1. Increased sphinganine levels and altered sphinganine/sphingosine ratios have also been observed in primary cultures of rat cerebrum that were treated with FB_1.[28] Monnet-Tschudi *et al.*[29] have used aggregating cell cultures of foetal rat telencephalon to study the effects of FB_1 on oligodendrocyte development and myelin formation. They noticed that FB_1 selectively affects glial cells, but not neurons. FB_1 is toxic in rat brain cell cultures, mainly due to drastically impaired myelin formation and myelin deposition, and due to delays in oligodendrocyte maturation.

As FB_1 may affect the central nervous system and induce neuronal damages through different mechanisms, we decided to explore the potential of FB_1 to cause oxidative stress. Hence the production of reactive oxygen species (ROS), lipid peroxidation, intracellular glutathione (GSH) levels, and cell viability were investigated in three different neural cell lines (human SH-SY5Y neuroblastoma, rat C6 glioblastoma and mouse GT1-7 hypothalamic cells).

MATERIALS AND METHODS

Materials

Human SH-SY5Y neuroblastoma cells, rat C6 glioblastoma cells and mouse GT1-7 hypothalamic cells were kind gifts from Dr. S. Påhlman (University of Uppsala, Sweden), Dr. N. Plesnila (University of Munich, Germany) and Dr. P. Mellon (University of California, USA), respectively. 2′,7′-Dichlorodihydrofluorescein diacetate, monochlorobimane, propidium iodide and digitonin were purchased from Molecular Probes Inc. (Eugene, OR, USA). Dulbecco's modified Eagle medium (DMEM), foetal bovine serum, trypsin, penicillin-streptomycin solution and Hank's balanced salt solution (HBSS; Ca^{2+} and Mg^{2+} free) were from Gibco (Paisley, UK). Phosphate buffered saline (PBS; Ca^{2+} and Mg^{2+} free) was from Orion diagnostica (Espoo, Finland). Protein assay reagents were from Bio Rad (Hercules, CA, USA), cell culture flasks from Nunc (Roskilde, Denmark), and multiwell plates from Costar (Cambridge, MA, USA). Fumonisin B_1, dimethyl sulphoxide (DMSO), ethylenediaminetetraacetic acid (EDTA), 2-thiobarbituric acid, 1,1,3,3-tetramethoxypropane (MDA) and phosphotungstic acid were from Sigma Co. (St Louis, MO, USA). All other chemicals used were of analytical grade.

Cell Culture and FB_1-treatment

In the study three different kinds of neural cell lines were used. The aim was to use different types of neural cells (neuroblastoma, glioblastoma and hypothalamic), which originated from human, rat and mouse. All cell lines were cultured in DMEM, supplemented with 10% inactivated foetal bovine serum, 50 U/ml penicillin and 50 μg/ml streptomycin, at 37°C in 5% CO_2 in an air-ventilated humidified incubator. The cells were grown in 75 cm^2 cell culture flasks and harvested either by 0.02% EDTA in PBS (SH-SY5Y cells) or by a solution containing 0.05% trypsin and 0.02% EDTA in PBS (C6 and GT1-7 cells). Either $1–2 \times 10^5$ cells in 0.5 ml or $2–3 \times 10^5$ cells in 1 ml medium were seeded on 48- or 24-well plates, respectively, and incubated for one to two days before starting the FB_1 exposures. FB_1 was diluted in DMSO and 5 or 10 μl of FB_1 solution was added to each well of the 48- or 24-well plates, respectively, to obtain final FB_1 concentrations of 0.01, 0.1, 1, 10 or 100 μM. Concentrations exceeding 100 μM were toxic, severely inhibiting the cell growth. The incubation times were 0.5, 1, 2, 3, 4, 5, 12, 24, 48, 72 or 144 h. Control cells were incubated for the same time periods with DMSO at a concentration of 1%.

Measurement of Cell Viability

Cell viability was measured with propidium iodide.[30,31] After incubating the cells with FB_1 on 48-well plates, the medium was removed and 50 μM propidium iodide in HBSS was added. After 20 min of incubation in the dark at room temperature, the fluorescence (F) was measured at an excitation wavelength of 530 nm and an emission wavelength of 590 nm using a Wallac Victor II Multilabel counter. Then, 160 μM digitonin was added for 20 min to permeabilize the cells and to cause 100% cell death. After permeabilization, the maximal fluorescence value (F_{max}) was measured. Background fluorescence was measured from cell free wells after the addition of 50 μM propidium iodide

and 160 μM digitonin ($blank_1$ and $blank_2$, respectively). The equation for calculating percentual viability[30] was:

$$\text{Viability}\,[\%] = 100 - [(F - \text{blank}_1)/(F_{\max} - \text{blank}_2)] \times 100.$$

Measurement of Reactive Oxygen Species (ROS)

The method used for measuring the production of ROS was modified from those described by McIntosh and Sapolsky[32] and Loikkanen *et al.*[31] The cells were cultured on 48-well plates, the medium was removed and the cells were loaded with 40 μM 2′,7′-dichlorodihydrofluorescein diacetate in HBSS for 20 min. In long-term experiments (12 h or longer), FB_1 incubations were carried out in DMEM at 37°C before addition of 2′,7′-dichlorodihydrofluorescein diacetate, and in short-term experiments in HBSS at room temperature after adding the probe. After loading, the cells were washed twice with HBSS. The formation of a fluorescent compound, 2′,7′-dichlorofluorescein, was monitored at an excitation wavelength of 485 nm and an emission wavelength of 535 nm using a Wallac Victor II Multilabel Counter (Wallac, Turku, Finland). Background fluorescence was determined from cell-free wells treated in the same way as the samples, and was subtracted from the fluorescence values of the cell samples. 2′,7′-Dichlorofluorescein values were divided with fluorescence values obtained after the addition of propidium iodide and digitonin ($F_{max;}$ described in the "Measurement of Cell Viability" section) to normalize the values to total cell number. Using normalized fluorescence values, the ROS production was calculated as percent of control at each time point.

Extraction and Determination of Malondialdehyde (MDA)-thiobarbituric Acid (TBA) Adduct

Cell incubations with FB_1 were carried out on 24-well plates, after which the medium was removed by centrifugation (10000 rpm, 3 min.). The cell pellets were re-suspended in 300 μl of SET buffer (0.1 M NaCl, 20 mM EDTA, 50 mM Tris–HCl, pH 8.0) and 20 μl of each sample was reserved for protein measurement.[33] Production of MDA in cell samples was used as an index of lipid peroxidation and oxidative cell damage. The method described by Baudrimont *et al.*[34] was applied for the formation and extraction of MDA-TBA adducts from the samples. An MDA standard curve (concentrations 3.9–125 nM) was prepared using a 250 nM 1,1,3,3-tetramethoxypropane solution in SET buffer, and the standards were treated in the same way as the samples. The MDA-TBA adducts were extracted from samples and standards with 300 μl of n-butanol

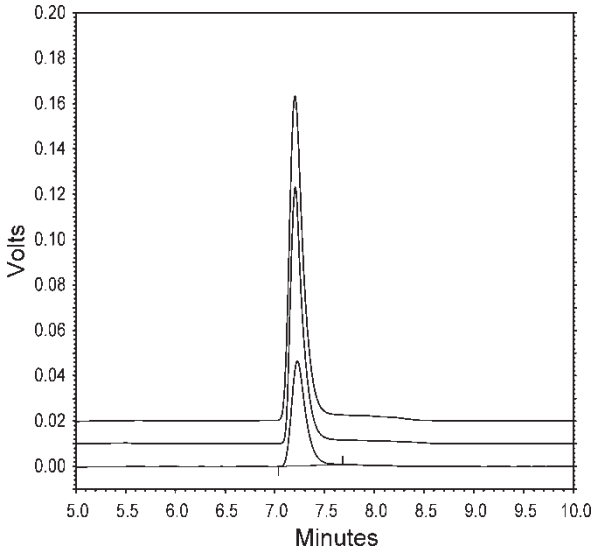

FIGURE 1 HPLC chromatogram of malondialdehyde–thiobarbituric acid (MDA–TBA) complexes. Bottom line: SET buffer (0.1 M NaCl, 20 mM EDTA, 50 mM Tris–HCl, pH 8.0); middle line: GT1-7 hypothalamic cells in SET buffer; upper line: 31 nM MDA-standard in SET buffer. Peak area of SET buffer was subtracted from areas of cell samples and standards when analysing the results.

before analyzed by HPLC and fluorometric detection. The HPLC system consisted of an LC-10AD pump, an SCL-10AVP system controller, an SIL-10A autoinjector, a RF-10AXL fluorescence detector and a CLASS-VP 5.032 chromatography software (Shimadzu, Duisburg, Germany). Twenty microlitres of each sample was injected into a Waters Spherisorb S3 ODS2 analytical column (4.6 × 150 mm) with a Waters Spherisorb S5 ODS2 guard column (4.6 × 30 mm). The mobile phase consisted of 20 mM KH_2PO_4–20 mM K_2HPO_4, pH 7.0 (eluent A) and methanol (eluent B). Analyses were performed using a binary gradient: 35% eluent B for 5 min, 35–70% B (5–7 min), 70% B (7–17 min), 70–35% B (17–18 min) and 35% B (18–28 min). The flow rate was 0.5 ml/min and the excitation and emission wavelengths were 515 and 544 nm, respectively. The areas of blank samples containing the SET buffer and treated in the same way as the cellular samples and standards, were subtracted from the peak areas of standards and samples. Concentrations of MDA in the samples were calculated by using linear 6-point MDA-calibration curves ($R^2 = 0.987–0.999$) obtained after subtracting areas of blank samples from peak areas of standards (Fig. 1).

Measurement of Intracellular Glutathione (GSH)

The levels of intracellular GSH were measured using a modified method of those described by Sarafian *et al.*[30] and Loikkanen *et al.*[31] The incubations with FB_1 were carried out in DMEM at 37°C,

if the incubation time was 12 h or longer. If the incubation time was 0.5–5 h, the medium was switched to HBSS before FB_1 was added at room temperature. After the incubations, the cells were washed with HBSS and loaded with 40 μM monochlorobimane in HBSS for 15 min. The formation of a fluorescent GSH–monochlorobimane complex was monitored at an excitation wavelength of 355 nm and an emission wavelength of 460 nm using a Wallac Victor II Multilabel counter. Cell free wells, treated in the same way as the samples, served as blanks and wells containing cells that were not treated with FB_1, served as controls. Fluorescence values of the blanks were subtracted from the fluorescence values of the cell samples. GSH–monochlorobimane fluorescence values were divided with fluorescence values obtained after the addition of propidium iodide and digitonin (F_{max}, see "Measurement of Cell Viability" section) to normalize the values to total cell numbers in each well. Using normalized fluorescence values, the levels of GSH were calculated as percent of control at each time point.

Statistical Analysis

Results are the mean ± SEM of four experiments, each done in quadruplicate. One-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test was used for statistical testing, and $P < 0.05$ was considered as statistically significant.

RESULTS

Cell Viability

We could observe a decrease in viability of SH-SY5Y cells after incubation with 100 μM FB_1 for 48–144 h. Treating GT1-7 hypothalamic cells with 100 μM FB_1 for 48–144 h lead to clearly decreased cell viability, as compared with the viability of control cells treated with DMSO (Table I). No significant changes in cell viability were observed at any of the earlier time points in these cell lines. FB_1 was not severely cytotoxic to C6 glioblastoma cells, because the viability of FB_1 treated cells did not statistically differ from that of the control cells (Table I).

Production of Reactive Oxygen Species (ROS)

In human SH-SY5Y cells, treatment with FB_1 did not affect production of ROS, as compared to controls, at any of the time points and at any of the fumonisin concentrations used (Fig. 2a).

An induction of ROS production was observed in mouse GT1-7 cells after treatment with 100 μM FB_1 for 72 and 144 h. The clearest effect was seen after 72 h, when ROS levels were 188% of control levels (Fig. 2b).

ROS production was significantly increased in rat C6 glioblastoma cells incubated with 100 μM FB_1 for 144 h. At earlier time points or with lower FB_1 concentrations, ROS production did not differ from that obtained in DMSO treated control cells (Fig. 2c).

Lipid Peroxidation

FB_1-induced lipid peroxidation was measured as an increase in the concentration of MDA. Increased MDA concentrations were seen in SH-SY5Y cells already after a 24-h incubation with 100 μM FB_1. After a 72-h incubation, increased MDA levels were observed in cells treated with 10 μM FB_1 (Fig. 3a). At the other time-points, MDA levels did not statistically differ from those of controls.

In GT1-7 cells, an increase in MDA levels was observed after incubation of the cells with 100 μM FB_1 for 144 h. Lower concentrations, or shorter incubation times, did not affect lipid peroxidation significantly (Fig. 3b).

The effects of FB_1 on C6 cells followed the same trend as with GT1-7 cells: incubation with 100 μM FB_1 for 72 and 144 h, but not for shorter incubation periods, induced lipid peroxidation as compared to control cells (Fig. 3c).

Intracellular Glutathione Levels (GSH)

Incubating cells with FB_1 clearly decreased GSH levels in all neural cell lines. In SH-SY5Y cells, decreased GSH levels (61% of controls) were

TABLE I Dose and time dependent effects of FB_1 on cell viability of SH-SY5Y, GT1-7 and C6 cells after 48–144 h of incubation

	SH-SY5Y cells			GT1-7 cells			C6 cells		
	Incubation time			Incubation time			Incubation time		
FB_1 conc. (μM)	48 h	72 h	144 h	48 h	72 h	144 h	48 h	72 h	144 h
0	92 ± 0.5	91 ± 2.1	94 ± 1.4	89 ± 0.3	86 ± 0.7	89 ± 2.3	91 ± 0.9	91 ± 0.5	86 ± 0.5
1	89 ± 1.8	84 ± 4.8	87 ± 3.3	91 ± 0.3	85 ± 1.8	90 ± 0.9	90 ± 1.3	90 ± 1.4	83 ± 1.6
10	88 ± 4.3	86 ± 3.7	88 ± 2.2	87 ± 0.8	78 ± 2.3	75 ± 5.1	87 ± 2.9	89 ± 2.3	88 ± 0.5
100	79 ± 2.6*	69 ± 6.8*	77 ± 4.6*	81 ± 2.4**	71 ± 2.6**	62 ± 6.9**	82 ± 5.0	88 ± 1.2	87 ± 1.9

Results are shown as percent of viability ± SEM. *$P < 0.05$, **$P < 0.01$ significantly different from control. No significant effects by FB_1 on cell viability were observed after incubation for 0.5–24 h.

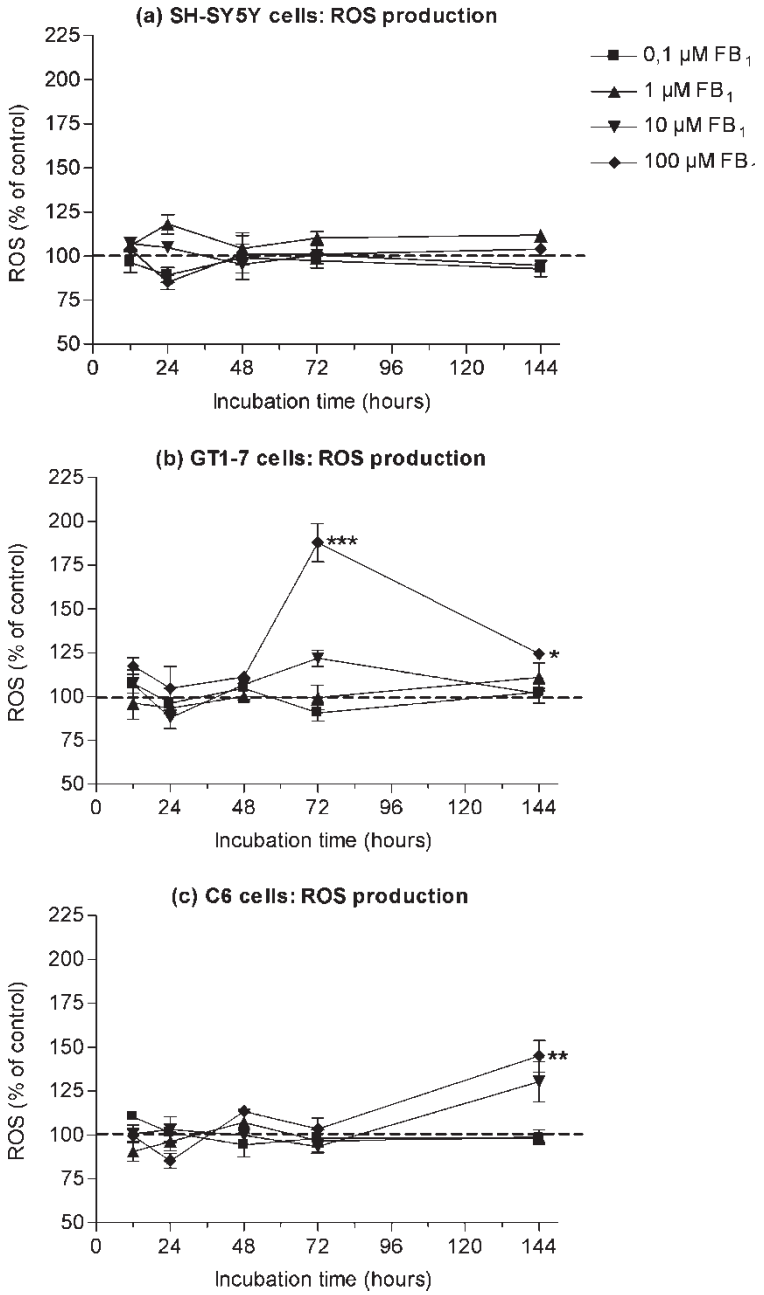

FIGURE 2 Effects of FB_1 on the production of reactive oxygen species (ROS) in (a) human SH-SY5Y neuroblastoma, (b) mouse GT1-7 hypothalamic and (c) rat C6 glioblastoma cells, exposed to 0.1, 1, 10 and 100 μM FB_1 for 12, 24, 48, 72 and 144 h. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ significantly different from control. No effects were observed after 0.5–5 h of incubation with FB_1 (results not shown).

observed after incubation of the cells with 100 μM FB_1 for 144 h, but not at earlier time points or with lower FB_1 concentrations (Fig. 4a).

Clear effects were seen in GT1-7 cells treated with 100 μM FB_1 for 72 and 144 h. In these cells, GSH levels decreased to 58% of control values after 144 h of incubation with FB_1. Decreased GSH levels were also seen in GT1-7 cells exposed to 1 and 10 μM FB_1 for 144 h (Fig. 4b). GSH levels in this cell line were not affected at earlier time points (0.5–48 h).

In C6 cells, significantly decreased GSH levels were observed after 48 and 72 h, when incubating cells with 10 and 100 μM, and 1 and 100 μM FB_1, respectively (Fig. 4c). Shorter incubation times or lower concentrations of FB_1 did not have any effect on GSH levels in this cell line.

DISCUSSION

Inhibition of ceramide synthase, followed by disruption of sphingolipid metabolism, seems to be the main mechanism underlying FB_1-induced toxicity.[17–19] Exposure to FB_1 may cause a central nervous system syndrome and equine leukoencephalomalacia.[4,5] Furthermore, *in vitro* studies

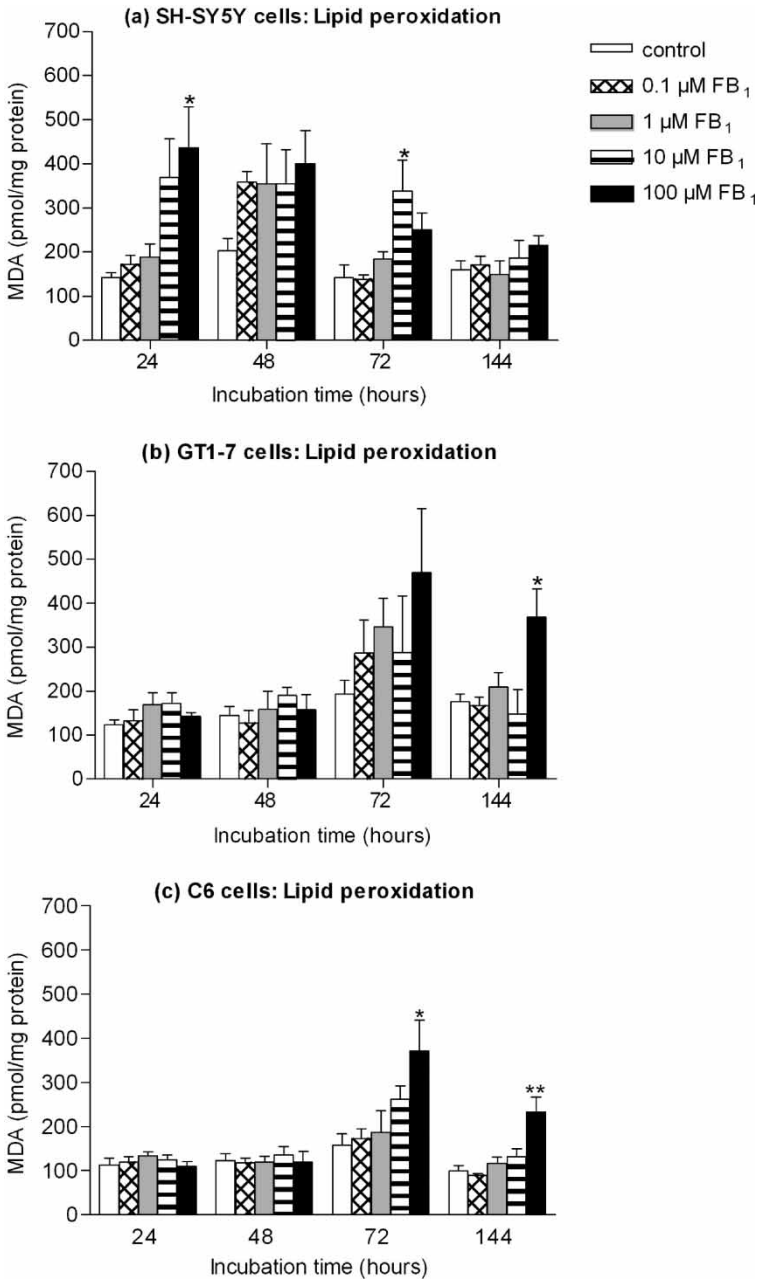

FIGURE 3 Lipid peroxidation measured as malondialdehyde (MDA) production in (a) human SH-SY5Y neuroblastoma, (b) mouse GT1-7 hypothalamic and (c) rat C6 glioblastoma cells, exposed to 0, 0.1, 1, 10 and 100 μM FB_1 for 24, 48, 72 and 144 h. *$P < 0.05$, **$P < 0.01$ significantly different from control. No effects were observed after 0.5–12 h of incubation with FB_1 (results not shown).

with different neuronal cells have shown that FB_1 causes cell death, induces apoptosis, inhibits protein synthesis and affects cellular sphingolipid levels.[24–26,28] Fusarium moulds are commonly found in samples of corn, but also in moisture-damaged buildings. In a study where fungi were isolated from samples of buildings, 10.6% of the houses were contaminated with *Fusarium* species. *Fusarium verticilliodes* was found both in air samples and in samples taken from the walls.[15,16] In a study by Tuomi *et al.*[13] 15% of bulk samples of mouldy interiors taken from moisture-damaged buildings contained *Fusarium* species.

In the present study, FB_1 (100 μM) was cytotoxic to GT1-7 and SH-SY5Y cells after 48, 72 and 144 h of incubation. FB_1 has also been shown to decrease cell viability of rabbit kidney cells,[35] human oesophageal epithelial cells and keratinocytes.[36] On the other hand, our results demonstrate that only incubations with a high concentration of FB_1 (100 μM) for 48 h induced a small decrease in the viability of C6 glioma cells. In other studies, FB_1 did not induce cell death or other signs of cytotoxicity in cerebral cell aggregate culture,[29] in rat astrocytes,[26] or in human fibroblasts,[37] which is in line with our results in C6 cells. However, contrary to our results,

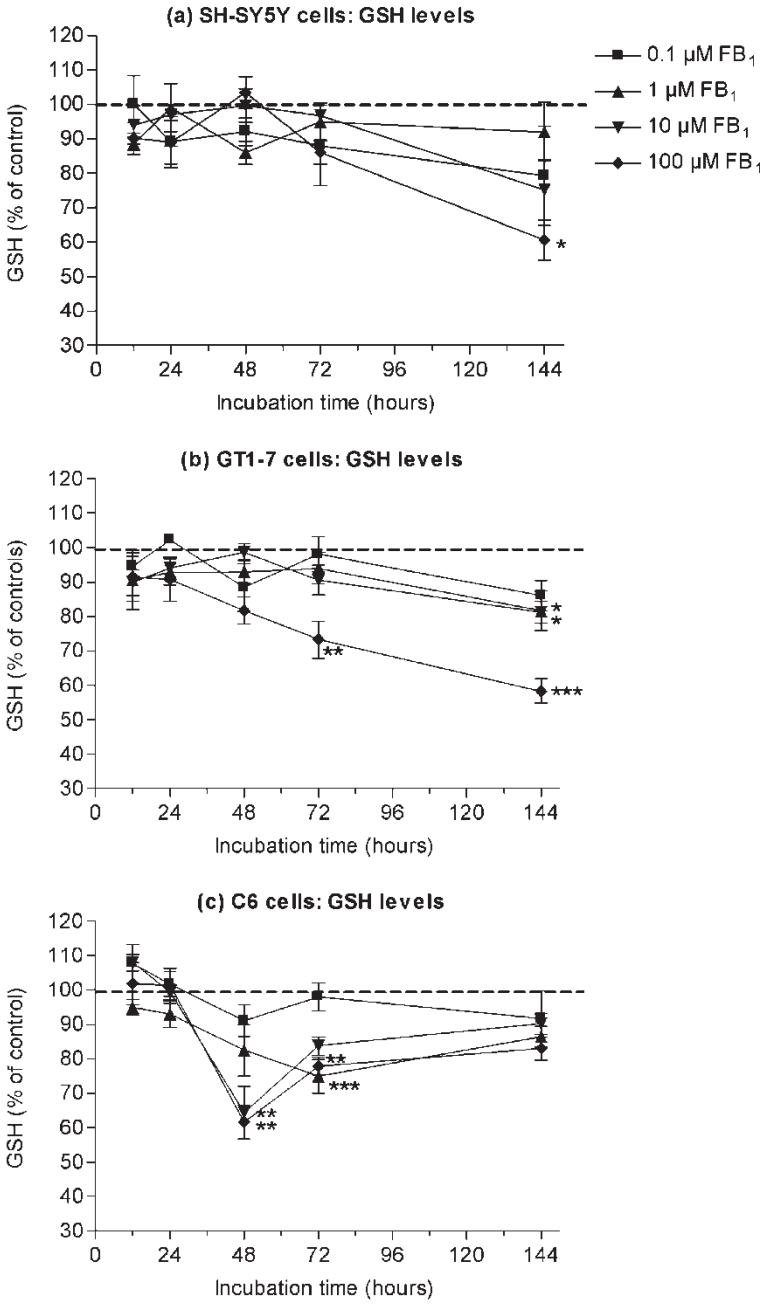

FIGURE 4 Effects of FB_1 on glutathione (GSH) levels in (a) human SH-SY5Y neuroblastoma, (b) mouse GT1-7 hypothalamic and (c) rat C6 glioblastoma cells, exposed to 0.1, 1, 10 and 100 μM FB_1 for 12, 24, 48, 72 and 144 h. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ significantly different from control. No effects were observed after 0.5–5 h of incubation with FB_1 (results not shown).

Mobio *et al.*[24] found that 9 μM FB_1 could decrease the viability of C6 cells already after 24 h, but that cell death could be prevented by a lipid-soluble antioxidant, vitamin E. The authors suggested that this confirms the involvement of lipid peroxidation in the cytotoxicity of FB_1. The difference between our results and those of Mobio *et al.*[24] may depend on the method used for the assessment of cell death, or on cell culture conditions.

The present study, supported by other *in vitro* studies,[38,39] indicates that FB_1 is cytotoxic to neural cells only at high concentrations *in vitro*. However, systemic toxicity, which may be caused by the inhibition of ceramide synthase, takes place already at very low concentrations of FB_1.[17] Therefore, direct comparison of cytotoxic doses of FB_1 *in vitro* with FB_1-induced *in vivo*-toxicity is problematic, and most likely is not relevant.

To our knowledge, effects of FB_1 on ROS production in different neural cell lines have not been reported before. In this study, FB_1 affected ROS production in different cell lines differently. In C6 cells, and particularly in GT1-7 cells, high doses of FB_1 clearly increased ROS production at late time

points, whereas in SH-SY5Y cells, production of ROS was not significantly altered by the FB_1 exposure. Thus, the ability of FB_1 to affect ROS production, and induce other events associated with oxidative stress, was cell line-specific. The reason for this observation remains obscure, but may be due to variable recognition of FB_1 by various cells or differences in sphingolipid metabolism. In other studies,[26,37] no effects on ROS production by FB_1 were observed in human fibroblasts, exposed to 10–100 μM FB_1 up to 72 h, or in primary cultures of rat astrocytes exposed to the same concentrations of FB_1 for up to 6 days. There was even a decrease in the production of H_2O_2 both in peritoneal cells of rats and in adherent peritoneal cells *in vitro* by FB_1, indicating a reduction of ROS production.[40] Theumer *et al.* have suggested that the decrease in ROS production in peritoneal macrophages may imply that FB_1 is an agent with immunosuppressive features, possibly causing decreased macrophage activity. This could be connected with decreased anti-tumour activity of these inflammatory cells.

In the present study, special attention was paid to the validation of HPLC-conditions to develop a quantitative method for an accurate assessment of lipid peroxidation. Indeed, increased lipid peroxidation was observed in all cell lines studied. On the contrary, in C6 cells exposed to 9–36 μM FB_1 for 24 h, Mobio *et al*.[41] observed clearly higher levels of lipid peroxidation than this study. These differences may be due to differences of experimental designs, or cell culture conditions. In addition, FB_1 also increased lipid peroxidation in rat hepatic cells and monkey kidney cells.[42,43]

In the present study, both ROS production and lipid peroxidation were increased in GT1-7 and C6 cells. These observations were consistent with the simultaneously decreased glutathione levels. It can be concluded that oxidative stress is an important mechanism whereby FB_1 affects these cells.

In a study on hepatic effects of FB_1, where no cytotoxicity was observed, it was suggested that lipid peroxidation occurred as a result of cell injury rather than being the cause of cytotoxicity.[44] Galvano *et al*.[26,37] have shown that FB_1 did not increase ROS production or cell death in human fibroblasts or rat astrocytes although DNA-damage and caspase-3 activation took place. Based on these results, the authors suggested that the effects of FB_1 are not a result of oxidative injury, but are instead a response that may occur after modulation of protective genes.[26,37] These theories support our observations in FB_1-treated SH-SY5Y cells. In these cells, lipid peroxidation took place without an increase in ROS production, and was associated with delayed cell death.

FB_1 clearly decreased GSH levels in each of the three cell lines studied, consistent with the assumption that FB_1 attenuated the defence against oxidative stress in these cells. The effects of FB_1 on GSH have not been studied in neural cells before, but it has been shown to decrease GSH levels in rat liver and spleen and also in murine liver cells.[45,46] However, in some studies, GSH levels even increased in mouse kidneys and in rat liver upon exposure to FB_1.[46,47] It is possible that FB_1, by disturbing the GSH defence system, renders cells devoid of effective protection mechanisms incapable of controlling consequences of the altered redox status. As a result, the cells may be unusually sensitive to radical attacks. Thus, even slightly increased production of various radicals could have a deleterious effect on the survival of these cells. Low expression of the anti-apoptotic Bcl-2 protein in GT1-7 and C6 cells can be linked to low basal GSH levels in these cell lines, and may increase their susceptibility to radical attack.[48–50] SH-SY5Y cells, on the other hand, express higher levels of Bcl-2,[50,51] which may explain why GSH levels in these cells decreased later than in GT1-7 and C6 cells exposed to FB_1.

In conclusion, our results show that FB_1 decreases intracellular GSH levels in different human and rodent continuous cell lines of neural origin. This event is associated with increased lipid peroxidation and ROS production, as well as decreased cell viability, all typical signs of oxidative stress. Therefore, we conclude that FB_1 is a potentially neurotoxic mycotoxin, and that oxidative stress plays a role in this effect of FB_1.

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