

Elevated sphingoid bases and their 1-phosphate for the toxic mechanism-based risk assessment of fumonisin as food contaminant

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Fumonisin are a family of mycotoxins produced by *Fusarium verticilloides* that are prevalent on corn, sorgham, millet and other agricultural products. There are currently several animal diseases and toxicities associated with fumonisins: equine leukoencephalomalacia (ELEM) in horses, porcine pulmonary edema (PPE) syndrome in pigs and liver cancer, hepatotoxicity and nephrotoxicity in rats and mice, and suspected to be involved in the occurrence of esophageal cancer in humans. Structurally similar to sphingoid bases, fumonisins are potent and specific inhibitors of ceramide synthase, a key enzyme in *de novo* sphingolipid biosynthesis and in the reacylation of free sphingoid bases. The specific objective was to determine the relationship among the changes in free sphingoid bases and their 1-phosphate concentrations and cell death upon fumonisin B1 (FB1) exposure. Most of FB1 toxicities can be explained by its ability to alter sphingolipid metabolism by inhibiting ceramide synthase. At least, an intracellular elevation of sphinganine (Sa) mediates the earliest toxicity of FB1. Some tissues such as kidney and liver may be most affected by FB1. In this study, FB1 was demonstrated to be accumulated not only Sa but also sphinganine 1-phosphate (Sa1P), which was typically observed in FB1-sensitive pig kidney epithelial cells (LLC-PK1 cells). Moreover, Sa1P was suggested to be a new indicator for FB1 exposure in rats and a diversity of culture cells, while sphingoid bases ratio was still useful in other organ tissues. The FB1 toxicity may be mainly mediated by endogenous Sa1P. In conclusion, FB1 sensitivity to sphingolipid metabolism is clearly related to the fumonisin toxicity and Sa1P appeared to be a sensitive biomarker for toxicity as well as risk management of fumonisins.

Elevated sphingoid bases and their 1-phosphate for the toxic mechanism-based risk assessment of fumonisin as food contaminant

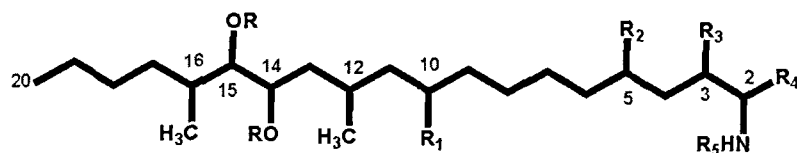


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Fumonisin: Chemical structure

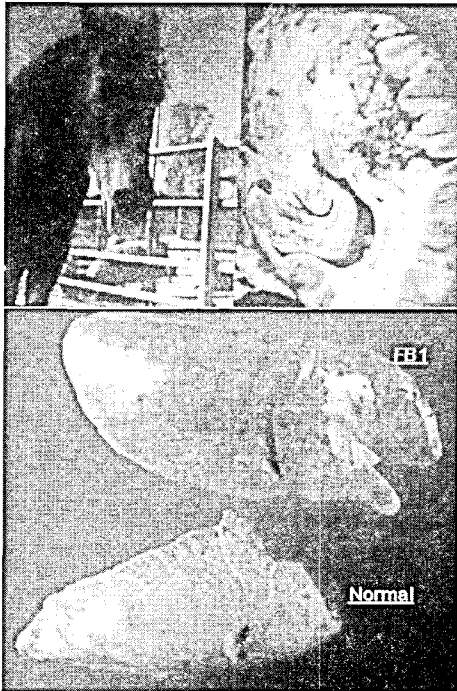


FB_1 : $\text{R}_1 = \text{OH}$, $\text{R}_2 = \text{OH}$, $\text{R}_3 = \text{OH}$, $\text{R}_4 = \text{CH}_3$, $\text{R}_5 = \text{H}$
 FB_2 : $\text{R}_1 = \text{OH}$, $\text{R}_2 = \text{H}$, $\text{R}_3 = \text{OH}$, $\text{R}_4 = \text{CH}_3$, $\text{R}_5 = \text{H}$
 FB_3 : $\text{R}_1 = \text{H}$, $\text{R}_2 = \text{OH}$, $\text{R}_3 = \text{OH}$, $\text{R}_4 = \text{CH}_3$, $\text{R}_5 = \text{H}$
 FB_4 : $\text{R}_1 = \text{H}$, $\text{R}_2 = \text{H}$, $\text{R}_3 = \text{OH}$, $\text{R}_4 = \text{CH}_3$, $\text{R}_5 = \text{H}$
 FA_1 : $\text{R}_1 = \text{OH}$, $\text{R}_2 = \text{OH}$, $\text{R}_3 = \text{OH}$, $\text{R}_4 = \text{CH}_3$, $\text{R}_5 = \text{CH}_3\text{CO}$
 FA_2 : $\text{R}_1 = \text{H}$, $\text{R}_2 = \text{OH}$, $\text{R}_3 = \text{OH}$, $\text{R}_4 = \text{CH}_3$, $\text{R}_5 = \text{CH}_3\text{CO}$
 FC_1 : $\text{R}_1 = \text{OH}$, $\text{R}_2 = \text{OH}$, $\text{R}_3 = \text{OH}$, $\text{R}_4 = \text{H}$, $\text{R}_5 = \text{H}$

Fumonisin toxicity

- Hepatotoxic in all species tested
- Nephrotoxic in many (JECFA PMTDI 2 ug/kg bw)
- Acutely toxic to horses (ELEM) and pigs (PPE)
- Carcinogenic to rats and mice (Group 2B)
- Promotes aflatoxin initiated liver cancer and co-occurs with AFB₁
- Suspected involvement in esophageal cancer in humans
- Mechanism = disruption of lipid metabolism

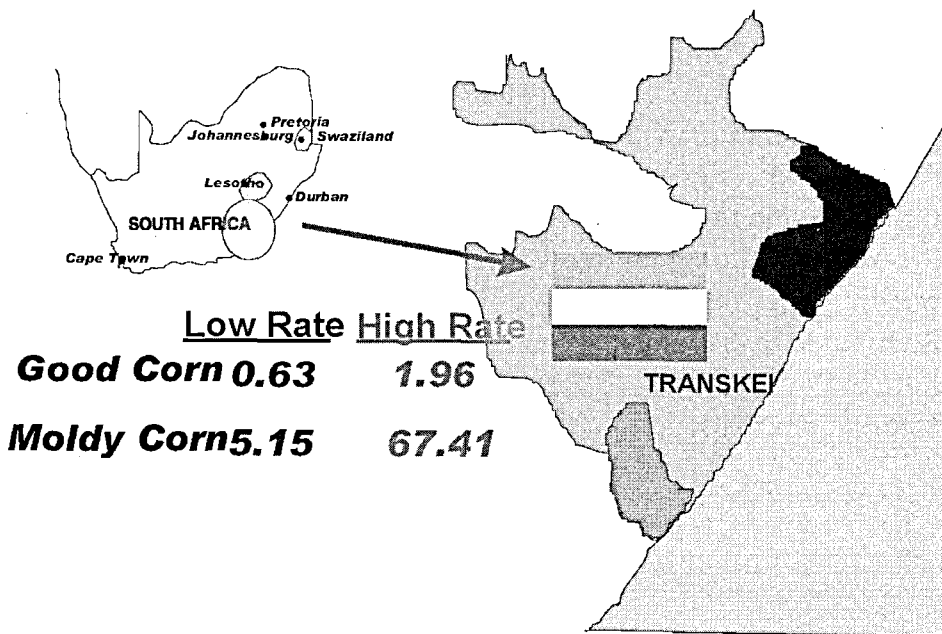
Fumonisin toxicity



ELEM in horse

PPE in swine

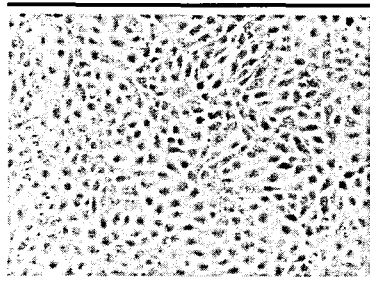
Association of fumonisins with esophageal cancer



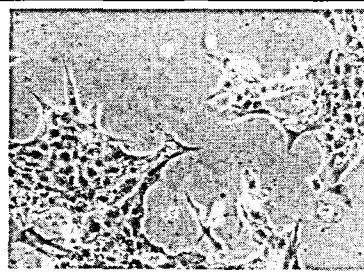
In vitro cytotoxicity

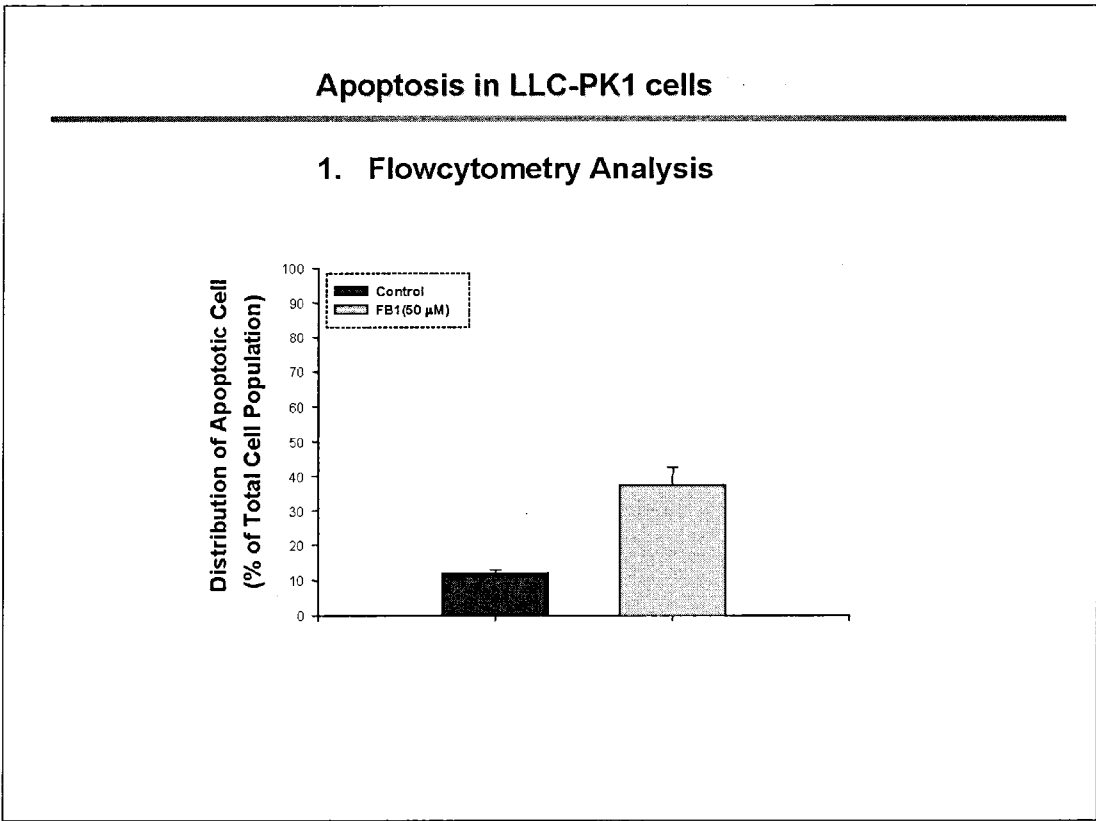
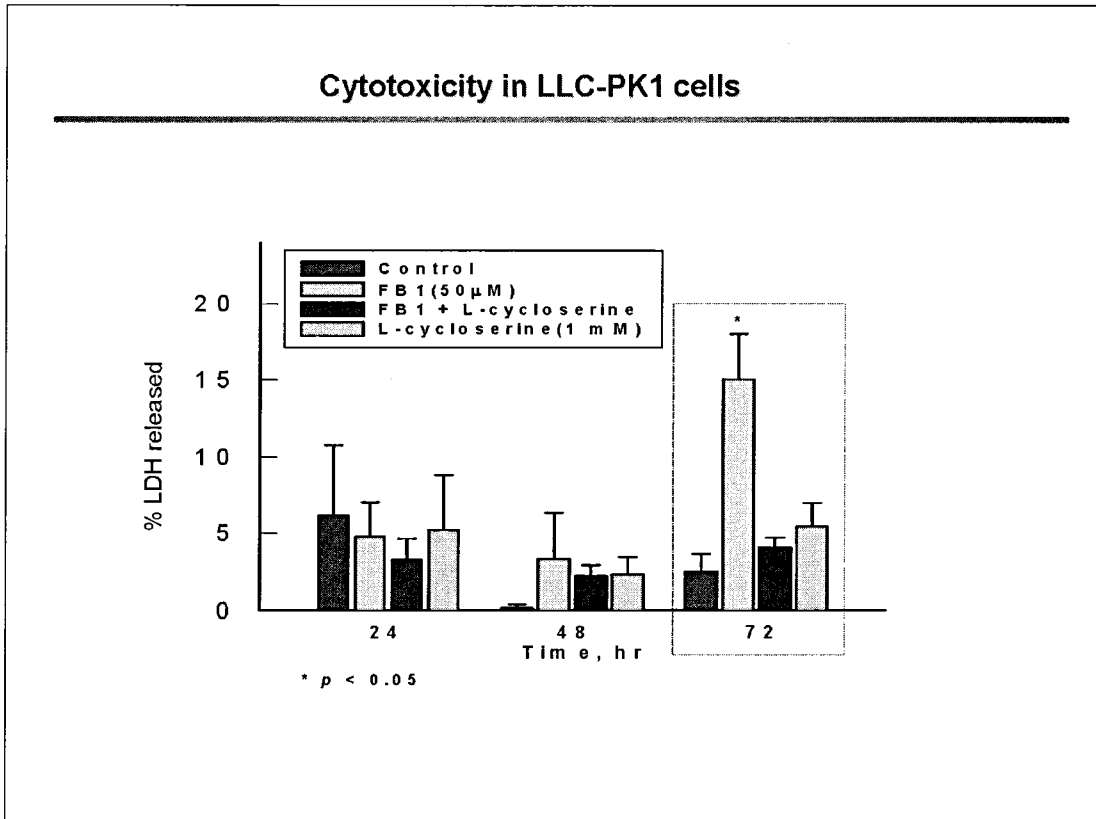
Morphological change in LLC-PK1 cells

Control



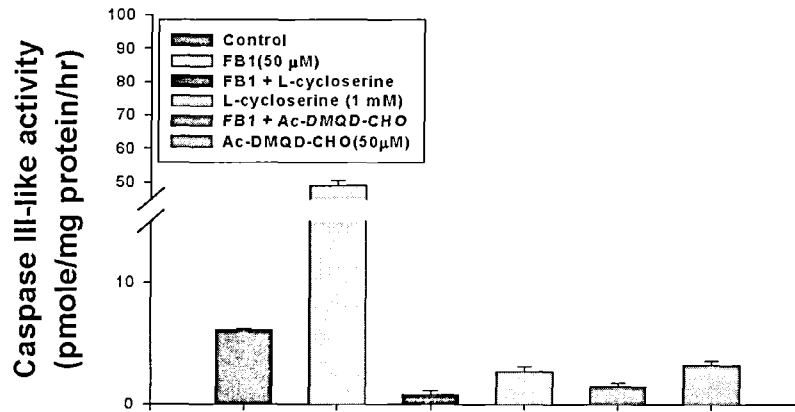
FB1(50μM)





Apoptosis in LLC-PK1 cells

2. Activation of Caspase III-like Activity

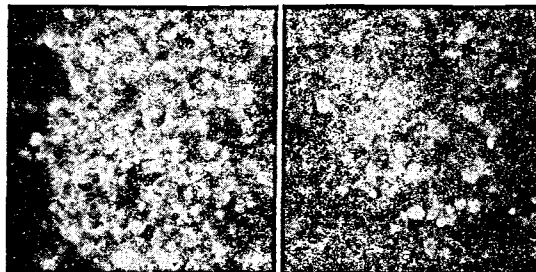


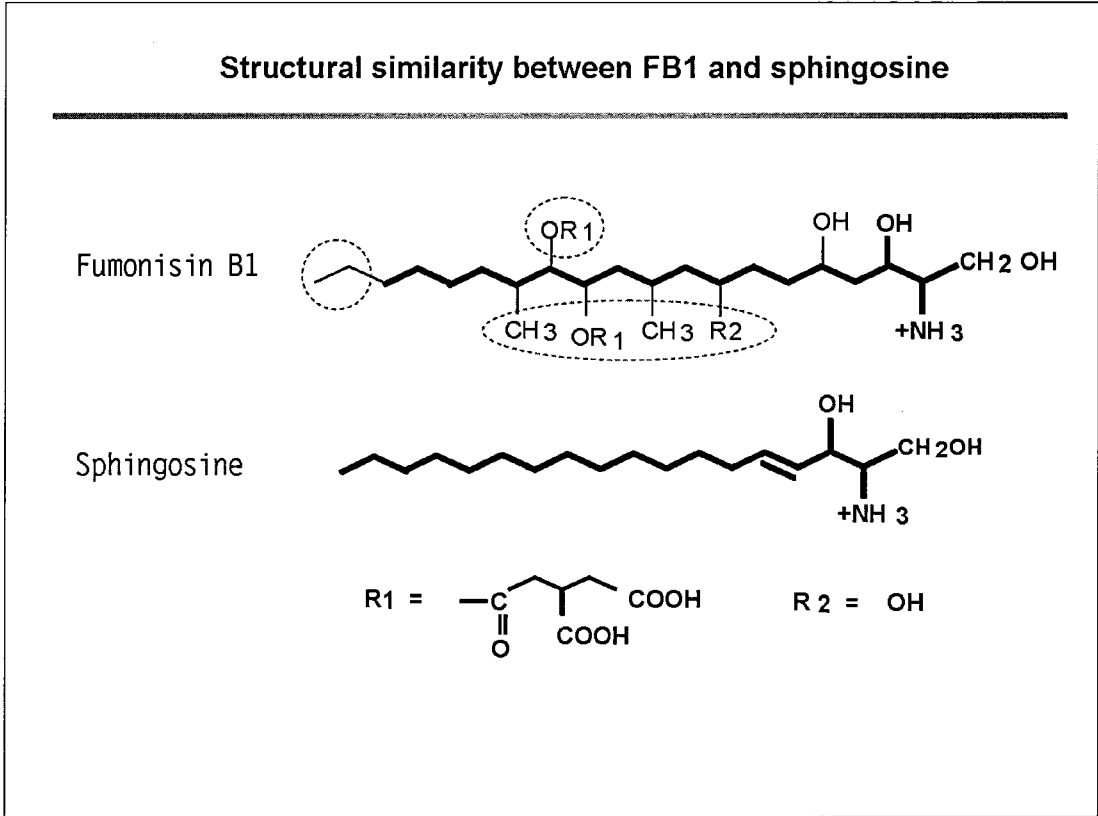
Apoptosis in LLC-PK1 cells

3. Confocal Microscopic Observation (TR-X phalloidin + FITC-BrdUTP)

Control

FB1(50 μM)






What are sphingolipids ?

- Physiologically bioactive compounds
- Present in all tissues and organs
- Approximately 300 sphingolipids
- Cell growth, differentiation, cell death

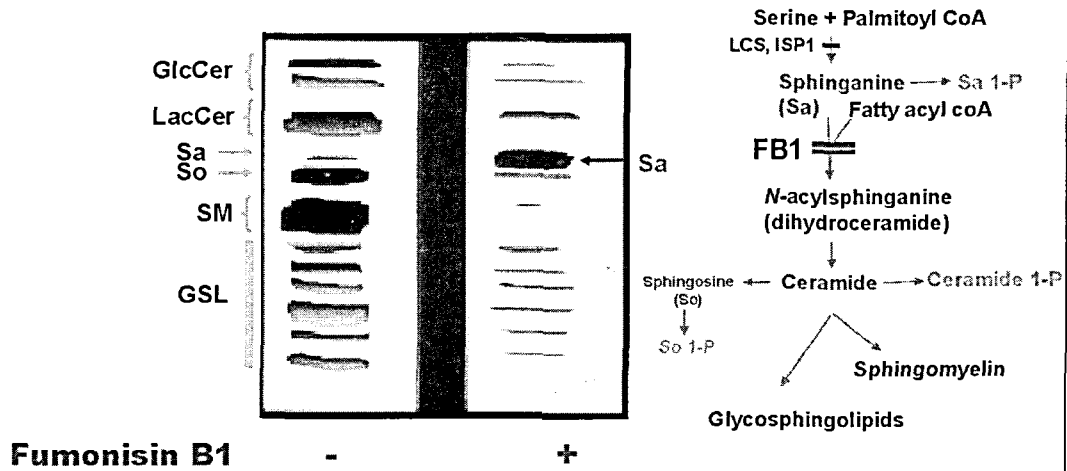
Glycolipid and Sphingolipid Biology
Guidelines Conference
January 27-February 1, 1992
Newport, Rhode Island, USA



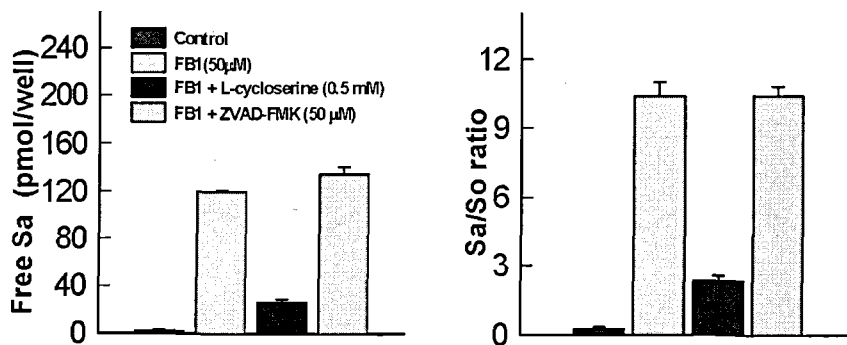
The following is a list of speakers at the conference. The names are listed in alphabetical order. The names of the speakers are listed in the order in which they spoke. The names of the speakers are listed in the order in which they spoke.

Altered *de novo* sphingolipids biosynthesis following FB1 exposure

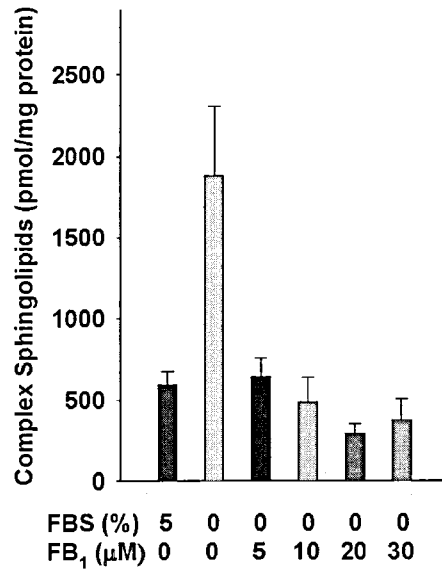
[¹⁴C]Serine incorporation into free sphingoid bases
incorporation into more complex sphingolipids



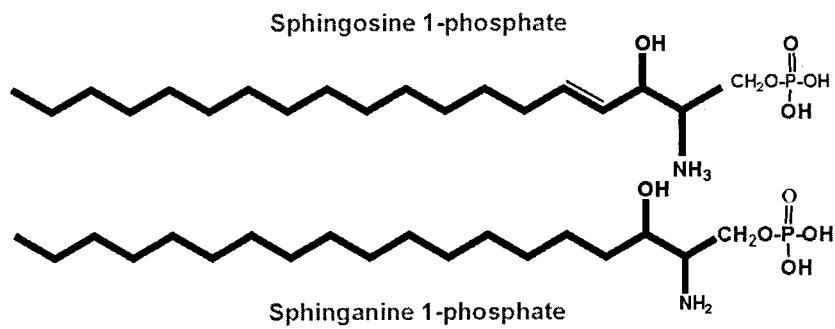
Intracellular Content of Free Sphinganine



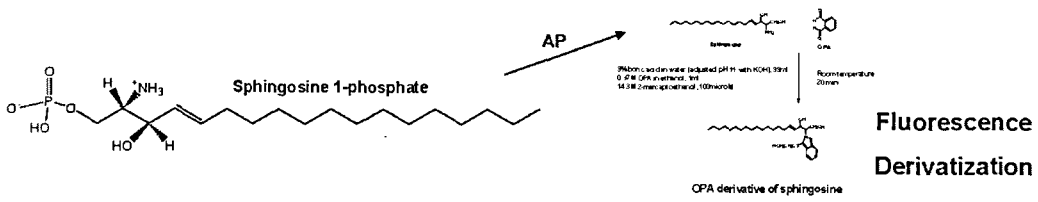
Intracellular Content of complex sphingolipids



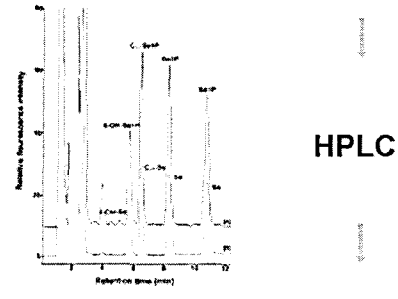
Chemical structures of sphingoid base 1-phosphate



Sphingoid base 1-phosphate analysis



Tissue (rat)	So1P	Sa1P	So	Sa
Eye	22.4 ± 4.8	11.8 ± 0.9	19.5 ± 3.0	57.9 ± 8.3
Heart	76.4 ± 5.5	11.2 ± 2.5	298.6 ± 3.8	35.1 ± 5.1
Intestine	51.2 ± 8.3	6.0 ± 2.7	186.6 ± 8.0	65.5 ± 3.0
Kidney	24.4 ± 3.5	4.1 ± 0.8	207.6 ± 7.7	32.4 ± 2.4
Liver	64.4 ± 5.9	13.1 ± 4.4		
Lung	81.4 ± 2.4	33.9 ± 1.5	106.6 ± 9.2	76.9 ± 4.1
Muscle	24.2 ± 4.1	25.1 ± 1.5	76.2 ± 6.7	17.1 ± 3.1
Spleen	545.4 ± 35.0	114.4 ± 7.4	419.5 ± 17.5	89.2 ± 1.8
Testis	27.4 ± 0.1	14.2 ± 0.5	105.8 ± 2.5	25.6 ± 0.3

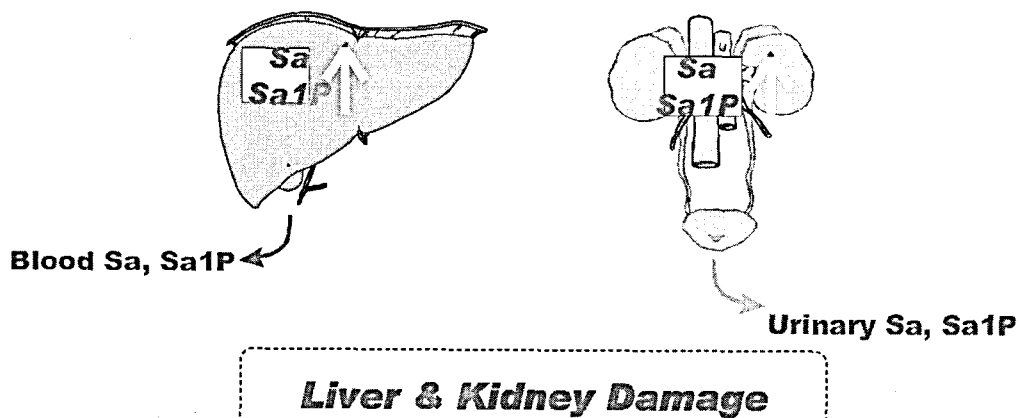


Sphingolipid: p mol/mg protein

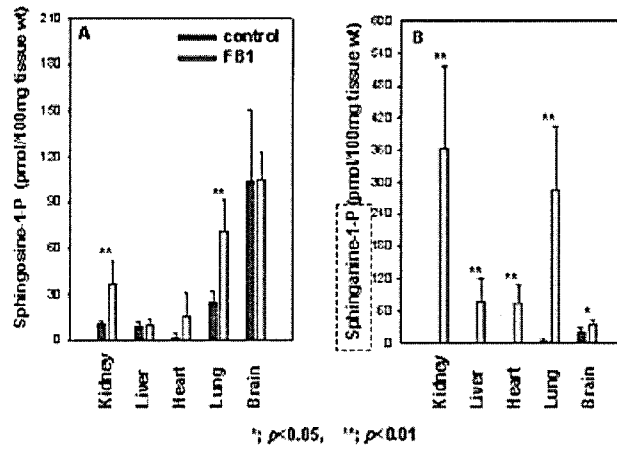
Pico mole sphingolipid analysis

In vivo toxicity

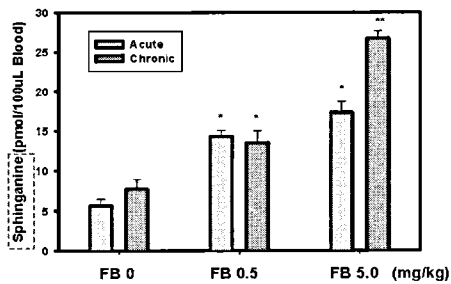
Ceramide synthase inhibition



Spingolipid alteration (tissues)



Spingolipid alteration (blood)

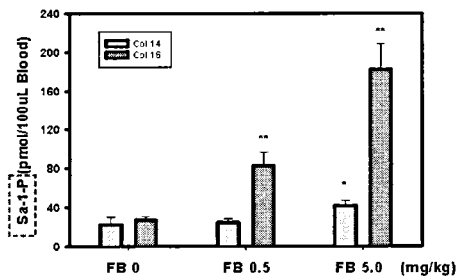


- Acute: single i.p. injection into mouse
 - Chronic: 5 day-injection

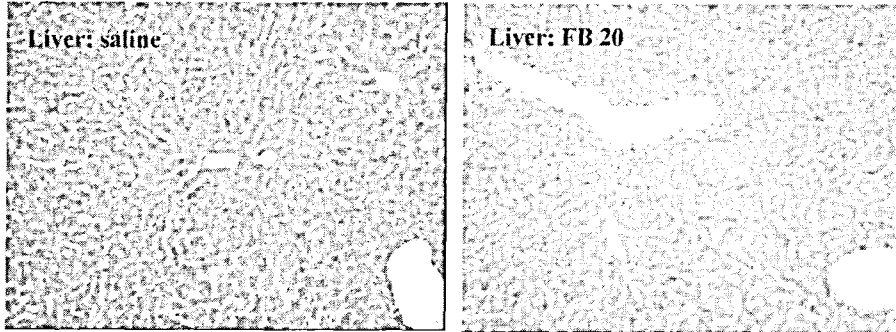
Spingolipid analysis (plasma)

- 1) Spinganine
- 2) Spinganine 1-phosphate

*p < 0.5, **p < 0.05 from the control

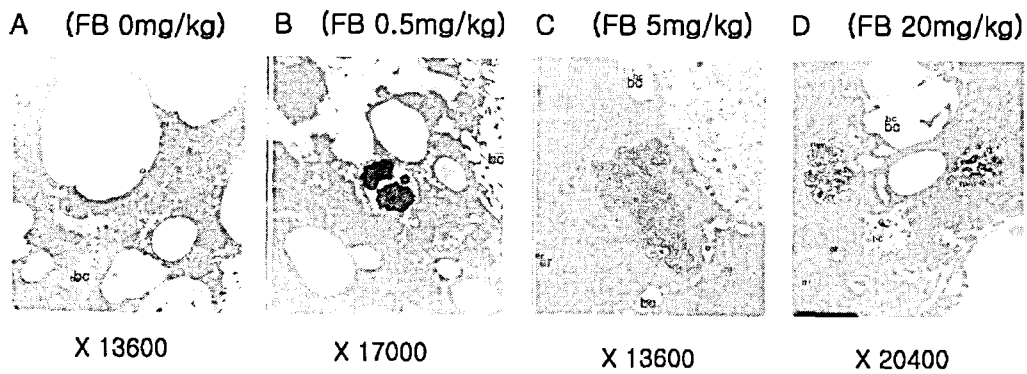


Hepatotoxicity (H/E staining)



Fumonisin을 5일간 20 mg/kg/day으로 처치한 후 liver와 kidney를 H/E로 염색한 후 light microscope에서 관찰한 결과 fumonisin처치군의 liver에서 binucleated cell 수가 뚜렷이 증가하고 acidophilic body의 출현으로 간세포독성이 확인

Hepatotoxicity (Electron micrograph)

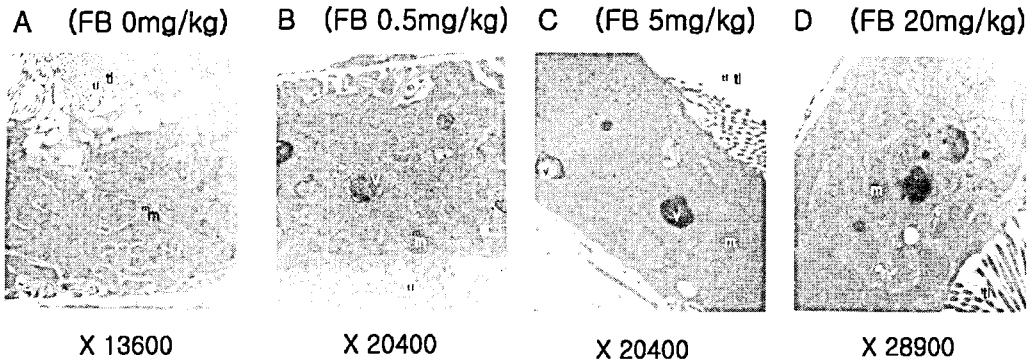


m: mitochondria
 mm: membranous material
 er: endoplasmic reticulum
 bc: bile canaliculi
 v: electron dense vacuole

Microscopic Observation

B: Electron dense vacuoles – degenerated lamella membranous mater
 C, D: Mitochondria – swelling, disrupted cristae
 Cytoplasm – accumulated lamellar membranous material
 ER – swelling, disruption
 Bile canaliculi – microvilli loss, lamellar membranous perforation

Renal toxicity (Electron micrograph)



m: mitochondria
tl: tubular lumen
v: electron dense vacuole

Microscopic Observation

B: Electron dense vacuoles – degenerated lamella membranous material
C, D: Mitochondria – swelling, disrupted cristae
Cytoplasm - lamellar membranous material accumulation

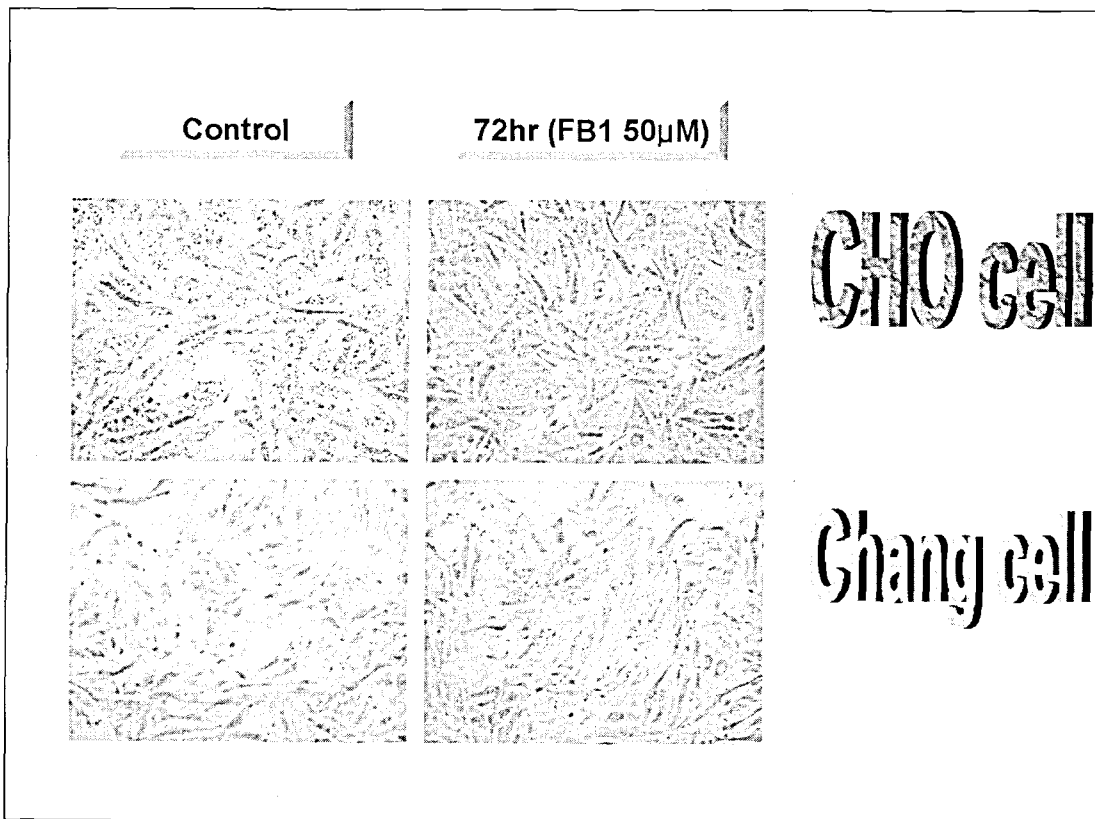
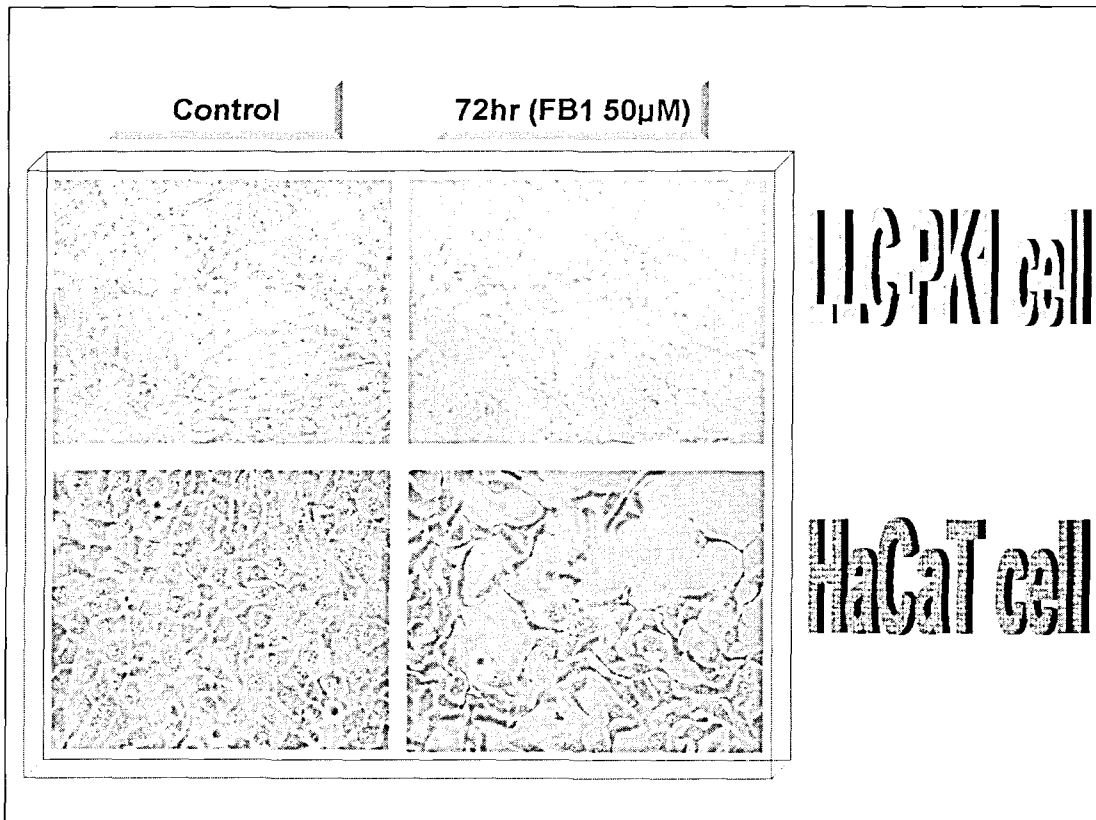
Fumonisin sensitivity for toxicity

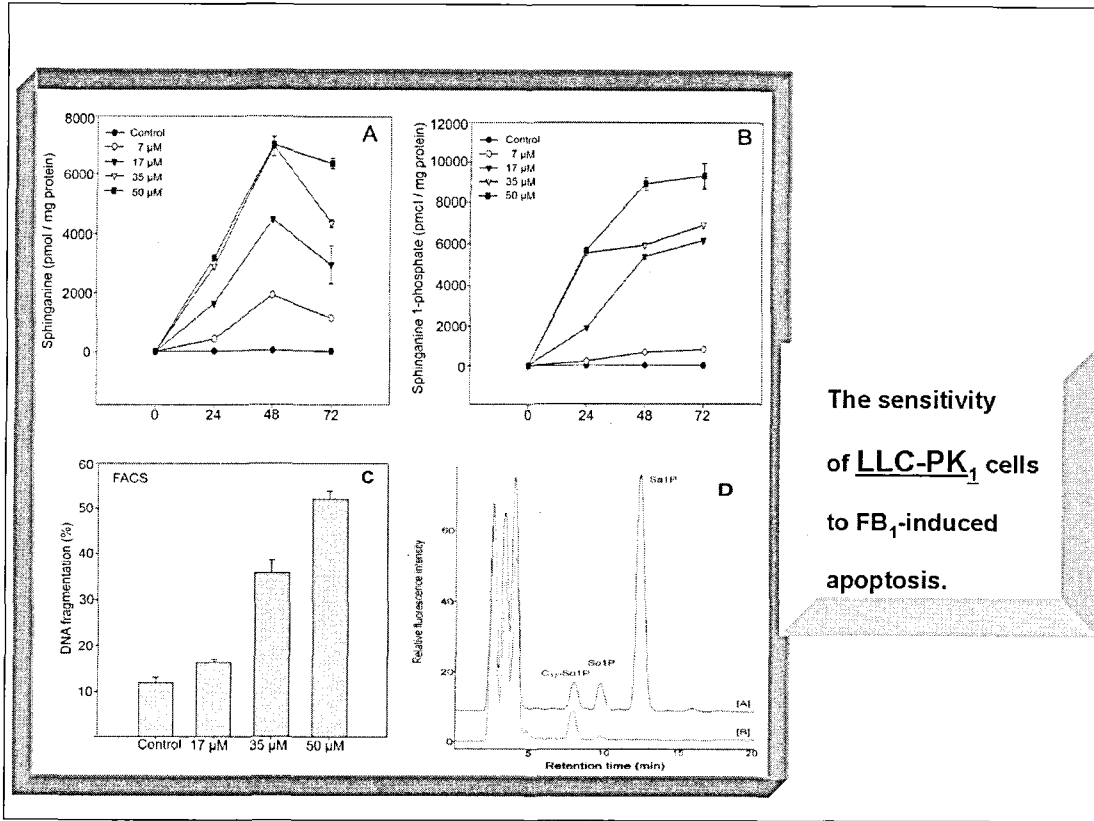
What causes the sensitivity difference of fumonisins among cell types ?

Differentiation between sphinganine and sphinganine 1-P for toxicity

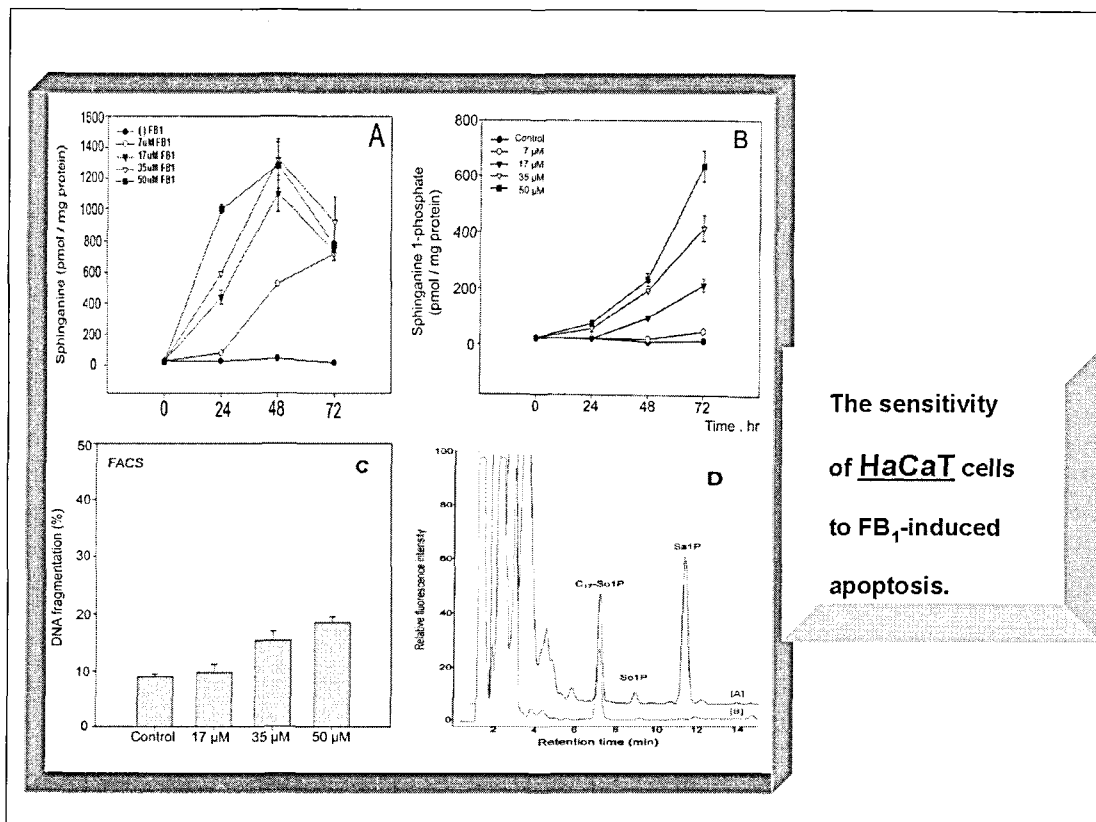


Cell death

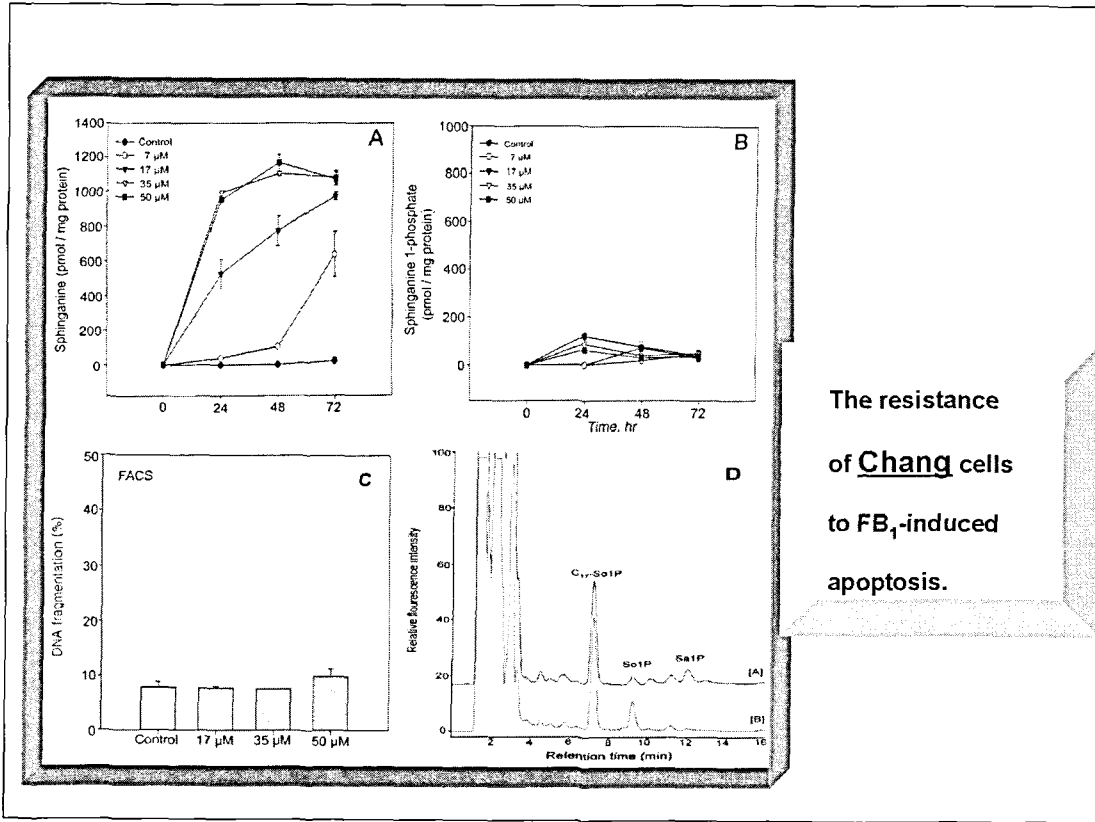




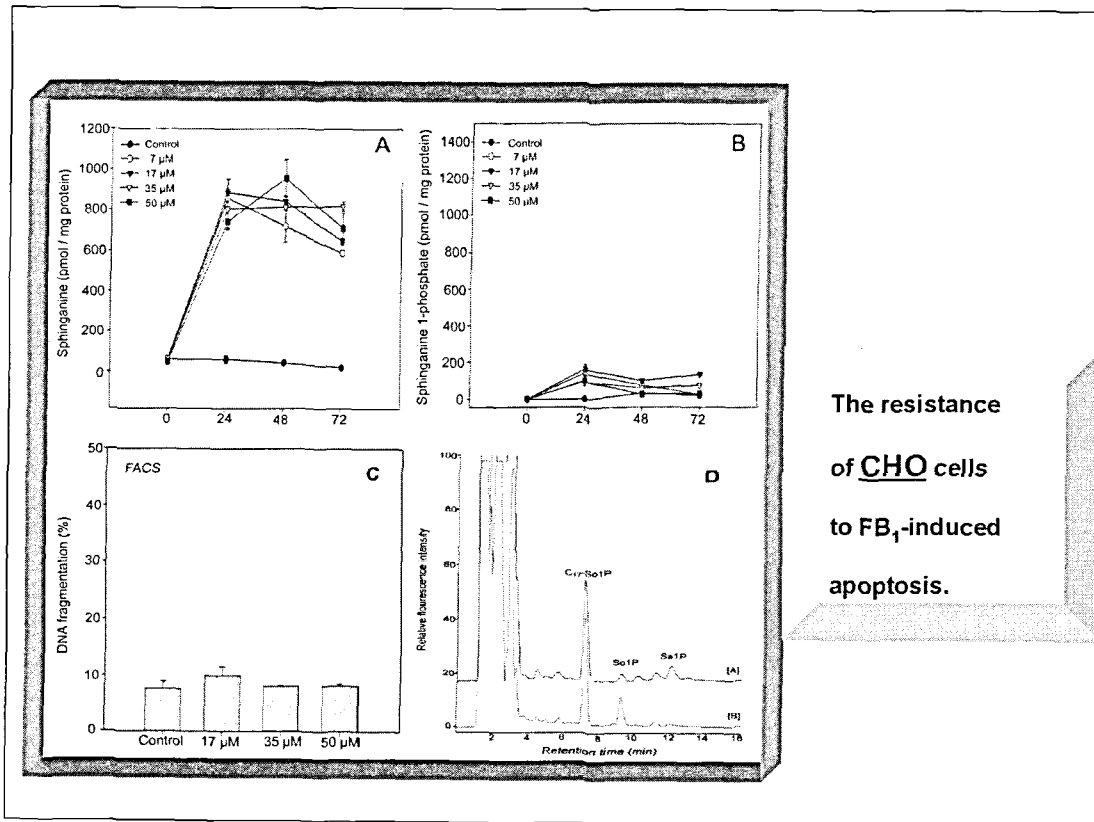
The sensitivity of LLC-PK₁ cells to FB₁-induced apoptosis.



The sensitivity of HaCaT cells to FB₁-induced apoptosis.



The resistance of Chang cells to FB₁-induced apoptosis.



The resistance of CHO cells to FB₁-induced apoptosis.

Conclusion

Fumonisin-induced toxicity appears to be related to (primarily) sphinganine 1-P and (to a lesser degree) sphinganine.

