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Trichothecin induces apoptosis of HepG2 cells via caspase-9 mediated activation of the mitochondrial death pathway

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ABSTRACT

Trichothecin, one of fungal toxins which were encountered in food and in the environment, seriously threatens human and animal health. It has been shown that trichothecin changed the morphology of cellular mitochondria. However, the molecular mechanism remains unknown. Here we found that cell viability was attenuated by trichothecin. Features of apoptosis such as homosomal condensation and inter nucleosomal fragmentation were observed. In consistence with the elevated apoptosis rate, expression of anti-apoptotic protein Bcl-2 was diminished and expression of proapoptotic protein Bax was enhanced at mRNA levels. Furthermore, expression of caspase-9 and activity of caspase-3 were increased after the treatment of trichothecin. Accordingly, the mitochondrial membrane potential ($\Delta\Psi m$) was decreased in a dose-dependent manner. And Ca^{2+} overload was induced by trichothecin, followed by the generation of reactive oxygen species (ROS). Collectedly, our results suggested that apoptosis induced by trichothecin is mediated by caspase-9 activation and the decrement of mitochondrial function resulted from the overloaded calcium and ROS production.

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1. Introduction

Contamination by mycotoxins in human food and animal feed is a severe worldwide problem (Rocha et al., 2005). Some mycotoxins are very frequently found in food, feed including processed grains, cereal products and other starchy materials, beans, peas, baking powders, barley, oats, cocoa, spices, wine and many other products. The UN Food and Agriculture Organization (FAO) pointed out that about 25% of world food production is contaminated by at least one mycotoxin (Heussner et al., 2006). Mycotoxins are secondary metabolites produced by microfungi that are capable of causing disease and death in humans and other animals (Sudakin, 2003). These mycotoxins are very stable during both storage/milling and processing/cooking of food (Bretz et al., 2006; Hazel and Patel, 2004).

Trichothecene mycotoxins share a 12,13- epoxy-trichothecene core structure and have been classified by their substitution pattern of specific side groups. Trichothecin possesses a ketone functional group at C8 (Fig. 1A) and it is classified as Type B trichothecenes (Rocha et al., 2005). It has been reported that trichothecin has a high infection rate in food and feed. Natural occurrence of trichothecin in wheat was reported with the infection rate ranging from 10% to 96% (Ishii et al., 1986). In addition, the presence of trichothecin in anise fruits, apples, and pears has been reported (Zabka et al., 2006).

Trichothecenes are now recognized as having multiple inhibitory effects on eukaryote cells, including inhibition of protein expression, DNA and RNA synthesis, and mitochondrial function(Minervini et al., 2004). Trichothecenes also affect cell division and membrane function(Bunner

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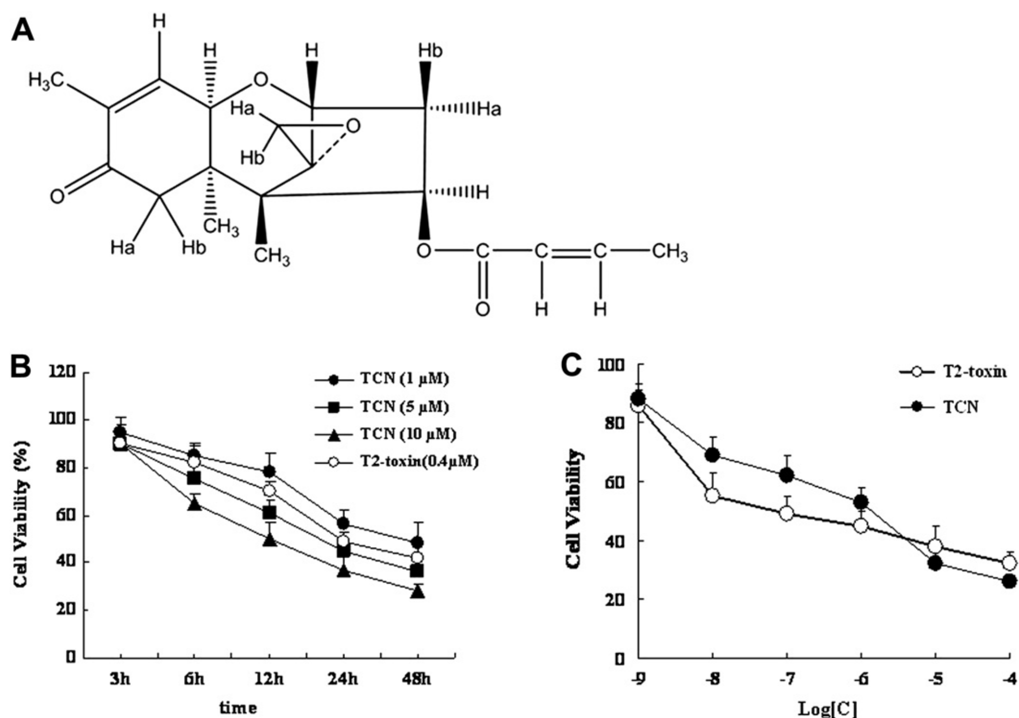

Fig. 1. A, Molecular structure of trichothecin. B, Cytotoxic effects induced by trichothecin (0.2, 1, 2 × IC_{50} corresponding to 1, 5, 10 μM) and T2-toxin (0.4 μM, 1 × IC_{50}) on HepG2 cells after 3 h, 6 h, 12 h, 24 h, and 48 h incubation. C, Cytotoxic effects of trichothecin and T2-toxin on HepG2 cells after 24 h incubation. The cell viability was determined by performing MTT assay and was shown as mean ± SD of eight determinations. T2-toxin was used as a positive standard control.

and Morris, 1988). In animal cells, trichothecene may induce apoptosis, a programmed cell death response, which plays an essential role during the development and homeostasis of most organisms (Osman et al., 2010).

Mitochondrial pathway was intrinsic pathway of apoptosis. Mitochondrial membrane permeabilization (MMP) is central integrators and coordinators of the apoptotic process (Mosmann, 1983). The loss of mitochondrial membrane potential ($\Delta\Psi m$) can result in the release of apoptogenic factors from the intermembrane space to the cytosol such as cytochrome c (cyt c), and promote caspases activation.

For trichothecin shares the common structure of trichothecene, supposedly, cytotoxicity of trichothecin is due to the apoptosis via mitochondrial pathway. John and his colleagues reported that trichothecin inhibited mitochondrial translation and affected mitochondrial morphology (McLaughlin et al., 2009). In this study we addressed the molecular mechanism of toxicity of trichothecin in vitro. Liver is the initial organ where the toxins are being metabolized and the concentration of the toxin is usually at the highest level after it is absorbed. Hence, HepG2 cells were selected as the in vitro experimental model to investigate the effects of trichothecin on apoptosis and apoptotic cascade.

2. Materials and methods

2.1. Materials

3-[4,5-Dimethylthiazol-2yl]-2,5-diphenyl tetrazolium bromide (MTT), T2-toxin and dimethyl sulphoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, MO). Trypsin, Dulbecco's-modified Eagle's medium (DMEM), fetal bovine serum, antibiotics (penicillin, streptomycin) and trypsin-EDTA solution were obtained from Gibco BRL-Life Technologies (Gibco, NY). All other reagents and solvents used were of analytical grade.

2.2. Cell culture

The HepG2 cell line was obtained from ATCC. The cells were cultured in DMEM supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal bovine serum (Gibco, NY), in a humidified 5% CO_2/95% air (v/v) atmosphere at 37 °C.

2.3. Cell viability assay

Cell viability was measured by the MTT assay based on the reduction of MTT by mitochondrial dehydrogenases of viable cells to a purple formazon product. HepG2 cells were plated in 96-well plates at a density of 5×10^3cells/well and grown for 24 h. After 24 h, culture medium was replaced by 100 μl serial dilutions of trichothecin (final concentration was 1, 5 or 10 μM, respectively) or T2-toxin (0.4 μM) and the cells were incubated for 3, 6, 12, 24 or 48 h. In addition, HepG2 cells were stimulated with trichothecin or T2-toxin at the dose of $10^{-9} \sim 10^{-4}$ M for 24 h. Then solutions with trichothecin or T2-toxin were aspirated and replaced by 200 μl DMEM without serum. Sterile filtered MTT stock solution in phosphate buffered

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