Induction of apoptotic cell death by trichothecenes

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Introduction

Trichothecenes are mycotoxins produced by various species of fungi such as Fusarium, Myrothecium, Trichothecium and Stachybotrys. Fusarium species are common and widespread in nature, and cause many harmful diseases such as alimentary toxic aleukia (ATA) and stachybotryotoxicosis in man and livestock. These mycotoxins induce cellular destruction in bone marrow and thymus cells, causing a reduction in peripheral white blood cell number. T-2 toxin (T-2), one of trichothecene mycotoxins, possesses a potent cytotoxicity to mammalian cells in vitro. In addition, the trichothecenes are potent inhibitors of eukaryotic protein synthesis by binding to the 60s ribosomal subunit. However, the detailed cytotoxic mechanism of trichothecenes is not elucidated. Recently, it has been evidenced that apoptosis, a physiological cell death observed during development and tissue turnover, is one of mechanisms of cytotoxicity induced by several chemical agents and anticancer drugs. We investigated the induction of apoptosis by T-2 and nivalenol (NIV) in vitro and in vivo.

1. Induction of apoptosis in HL-60 cells by trichothecenes

Apoptosis, morphologically distinguishable from necrosis and characterized by condensation of cytoplasm, loss of plasma membrane microvilli, and fragmentation of chromatin DNA, occurs during development and tissue turnover. Based on the DNA fragmentation profile and the morphological changes, the trichothecenes including T-2, NIV, deoxynivalenol (DON), roridin A (RA) and satratoxins (ST) induced the apoptosis in human promyelocytic cell line HL-603. Especially, T-2, RA and ST are potent inducers of apoptosis. From morphological observation, T-2 induces apoptotic morphological changes including loss of microvilli, marked chromatin condensation, and karyorrhexis.

2. Intracellular events in T-2-induced apoptosis

The physiological control of intracellular calcium ion (Ca++i) level is a critical event in proliferation, differentiation and apoptosis. To elucidate the kinetics of Ca++i in T-2-induced apoptosis, Ca++i level was investigated using confocal laser microscopy2. Prior to apoptosis,

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Ca++i level was markedly elevated within 3-5 min after exposure to T-2 and returned to control level thereafter. Generally, as reported in glucocorticoid-induced apoptosis, Ca++i level is continuously elevated during apoptosis. This is the first report that a transient elevation of Ca++i is occurred in the induction of apoptosis.

On the other hand, T-2-induced apoptosis is completely blocked by pretreating with a chelator of Ca++i, BAPTA-AM or a Ca++i-dependent endonuclease inhibitor, ZnCl₂. These results indicate that the mobilization of Ca++i is indispensable in T-2-induced apoptosis.

Recently it was cleared that the apoptotic signal cascade is mediated by caspases, a family of cystein protease, and the disruption of mitochondrial transmembrane potential (ΔΨm), followed by cellular and nuclear morphological changes. In T-2-induced apoptosis, caspase-3-like proteases were activated 1 hr after the exposure (Fig. 1), and T-2-induced apoptosis was completely blocked by pretreating with 80 μM of z-VAD-fmk, an inhibitor of caspases. In addition, the disruption of ΔΨm was markedly occurred 1 hr after the exposure (Fig. 2). The maintenance of ΔΨm is partly performed by Bcl-2 family proteins. However, the expression of

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**Fig. 1** Activation of caspase-3-like proteases during T-2-induced apoptosis. HL-60 cells were treated with 100 ng/ml of T-2 for the times indicated, and activity of caspase-1- and caspase-3-like proteases was estimated using the synthetic substrates, Ac-YVAD-MCA and Ac-DEVD-MCA, respectively. Activity of caspases was quantified by measuring MCA release. One unit equals 1 pmol of AMC released.

**Fig. 2** The disruption of ΔΨm by T-2. HL-60 cells were cultured in the absence (A) or presence (B) of 100 ng/ml of T-2 for 1 hr. The ΔΨm was evaluated by the uptake of the ΔΨm-sensitive dye, DiOC₆(3). Control cells were simultaneously treated with the protonophore mCICCP.
Bcl-2 was not altered. The disruption of $\Delta \Psi_m$ by T-2 may be mediated by other intracellular factors.

These results strongly indicated that the signal transduction pathway primed by T-2 is mediated by the common pathway of apoptosis recognized by FasL, several anticancer drugs and other apoptotic stimuli.

3. **Apoptotic cellular damage in mice after trichothecenes-induced acute toxicosis**

Trichothecenes are potent inducers of apoptosis in mammalian cell line in vitro. From numerous toxicological studies in mice and rats, the trichothecene mycotoxins possess a potent activity to induce malfunction of hematopoietic organs, characterized by a marked decrease in numbers of circulating white blood cells, necrotic lesions and karyorrhexis of actively growing cells in bone marrow, thymus and spleen, and impairment of immunologic response. By histopathologic, electron microscopic and immunohistochemical observations, the mechanism of cellular death was investigated in thymus and spleen of mice given T-2 or NIV. In the thymus and spleen of mice given 5.0 mg/kg body weight of T-2 and killed 2 hr later, a massive cellular destruction characterized by chromatin condensation was evident, and electron microscopy analysis revealed the presence of apoptotic bodies. In situ nick translation analysis (TUNEL method) revealed DNA fragmentation in thymus and spleen. The same findings were observed in the thymus and spleen of mice given 8.2 mg/kg b. w. of NIV. These findings indicated that trichothecenes such as T-2 and NIV are potent inducers of apoptosis in vivo. Moreover, from these observations, we proposed a novel morphological feature of apoptosis that the crescent-shaped spaces (CSS) are recognized around the nuclear envelopes of cells at a quite early stage during apoptotic cell death.

4. **Induction of apoptosis in human peripheral blood lymphocytes by the trichothecenes**

It is well known that repeated administration of T-2 and related trichothecenes to animals decrease severely the number of circulating white blood cells. Furthermore, it was cleared that trichothecenes are potent inducers of apoptosis in vitro and in vivo. To elucidate a possibility of direct effects of T-2 and NIV on peripheral blood cells causing a reduction in the number of peripheral blood cells, we analyzed T-2 or NIV-induced apoptosis in human peripheral blood lymphocytes (hPBLs) in vitro. These toxins induced apoptosis in hPBLs, and, by flow cytometric analysis, this apoptosis was observed in lymphocyte subsets including T cell, B cell, natural killer cell, activated cell, resting cell, naive cell and memory cell. In addition, T-2 and NIV induce apoptosis in hPBLs through Ca$^{++}$i-mediated multiple signal transduction pathways, since T-2- or NIV-induced apoptosis were strongly inhibited by pretreating with BAPTA-AM.

These data suggest that the trichothecenes directly affect the hPBLs, and the elicited serious apoptotic cell death causes, in part, a marked decrease in circulating white blood cells, as observed in trichothecene-administered animals.

5. **Conclusion**

As a whole, the trichothecenes are potent inducers of apoptosis in HL-60 cells and hPBLs.
Recently, the signal transduction pathway of apoptosis in Fas-FasL system has been rapidly clarified. Furthermore, although apoptosis is essential to normal homeostasis, the disturbance of apoptosis induces several severe diseases including acquired immunodeficiency syndrome (AIDS) and Alzheimer's disease. Although the elucidation of apoptotic signal transduction pathway was mainly evidenced in a common pathway including the dysfunction of mitochondria, DNA endonuclease, caspases and its substrates, an early event of apoptosis, namely an individual pathway, has not yet been elucidated. T-2 induced a transient elevation of Ca\(^{++}\), and this phenomenon is essential to the induction of apoptosis by trichothecenes. Investigation on the detailed mechanism of mobilization of Ca\(^{++}\) by the trichothecene is expected to solve an intracellular event of early stage of apoptosis.

On the other hand, the trichothecenes induced the apoptotic cell death in the thymus and spleen. It is well known that T cell precursors differentiate into immunocompetent T cells within the thymus, and mature immunocompetent cells including T cell, B cell and macrophage recruit into the secondary lymphoid organs including spleen. The apoptotic cell death was observed in these organs, and the trichothecenes nonspecifically induced apoptosis in hPBLs in vitro. These findings propose a hypothesis that the immunosuppressive effect of trichothecenes is partly elicited by a direct induction of apoptosis in the immunocompetent cells.

References