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学 位 論 文 名	T-2 toxin initially activates caspase-2 and induces apoptosis in U937 cells (T-2 毒素はカスパーゼ 2 を活性化することにより、U937 細胞のアポトーシスを誘導する)
論 文 審 査 委 員	(主査) 教 授 森本 兼曩 (副査) 教 授 長田 重一 教 授 磯 博康

論 文 内 容 の 要 旨

[目 的]

T-2 toxin is one of the type A trichothecene mycotoxins, produced by several fungal genera including *Fusarium* species. It is detected in a number of field crops (wheat, maize, barley and oats) and processed grains (malt, beer and bread). Both humans and animals suffer from several pathologies due to intoxication after consumption of foodstuffs contaminated with T-2 toxin and other trichothecenes. Studies showed that T-2 toxin induces severe damage in bone marrow, lymph nodes, spleen, thymus, and intestinal mucosa, resulting in adverse effects such as lymphopenia and immunosuppression in many species. The underlying mechanism of these changes was found to be apoptosis. T-2 toxin was shown to inhibit mitochondrial function, protein synthesis, inhibit mitochondrial succinate dehydrogenase activity and increases mitochondrial NADH dehydrogenase activity. It also induced the release of cytochrome c from mitochondrial intermembrane space into the cytosol. But the mechanism of T-2 and other trichothecenes induced apoptosis was not clear. The study addressed the toxicity mechanism and death signaling of T-2 toxin with special emphasis on the specific role of caspase-2 and its downstream events.

[方 法]

We used the human monocytic leukemia U937 cell line to investigate the mechanism of T-2 toxin-induced apoptosis. At first, WST-1 assay was used to test toxicity of T-2 toxin. To test T-2 toxin-induced apoptosis, DAPI staining for nuclear fragmentation, internucleosomal DNA fragmentation assay and caspase activity assay were performed. We used 10^{-8} and 10^{-7} M T-2 toxin to determine the order of caspase activation in T-2 toxin-induced apoptosis. Mitochondria were isolated from U937 cells to test if mitochondria respiratory chain complexes were inhibited in this process. Caspase-9, -2, and -8 inhibitor was preincubated with the cells for 2 hours and then T-2 toxin-induced caspase-3 activity, the process of procaspase-3 to its active form of 19 kDa were analyzed to make clear which caspase is essential for T-2 toxin-induced apoptosis. Both enzyme activity assay

and western blot analysis were used to test which caspase was the first activated and was apical in T-2 toxin-induced apoptosis.

[成 績]

T-2 toxin induced apoptosis with distinct morphological and biological features in U937 cells as determined by DAPI staining, DNA fragmentation and caspase-3 activity assay. The concentration of more than 10 nM T-2 toxin affected cell viability, induced nuclear and DNA fragmentation and caspase-3 activation. Caspase-2, -3, -8, and -9 were activated during T-2 toxin-induced apoptosis. T-2 toxin neither inhibited mitochondrial respiratory chain complexes I to IV in isolated mitochondria nor decreased ATP levels in U937 cells. Both enzyme activity assay and western blot analysis revealed that T-2 toxin activated caspase-2 earlier than caspase-3, -8, and -9. Caspase-2 inhibitor (VDVAD-CHO/fmk) and caspase-8 inhibitor (IETD-CHO/fmk) completely blocked the T-2 toxin-induced process of procaspase-3, while caspase-9 inhibitor (LEHD-CHO/fmk) did so less effectively. Caspase-2 inhibitor entirely blocked T-2 toxin-induced caspase-8, and -9 activation while caspase-2, -3 -8, and -9 inhibitor did not show any effect on T-2 toxin-induced the process of procaspase-2.

[総 括]

T-2 toxin from 10^{-8} to 10^{-5} M inhibited U937 cell proliferation and induced apoptosis dose dependently. Initiator caspase-2 is involved in T-2 toxin-induced apoptosis. During the apoptosis, caspase-2 and -8, -9, and -3 were activated and activation of caspase-2, and -8 is required for the process of caspase-3 activation, while caspase-9 activation might function as an amplifier for the apoptosis. Therefore, the activation of caspase-2 is essential to T-2 toxin-induced apoptosis as determined by caspase-3 activation, and apoptotic signals are mainly transmitted via caspase-8 rather than the mitochondrial pathway. These findings will help alleviate T-2 toxin induced cytotoxicity and relevant disorders.

論文審査の結果の要旨

T-2 毒素は赤カビから生産され、様々な食品の中に検出される真菌毒素 (trichothecenes) である。T-2 毒素によって、免疫細胞死で免疫抑制が、T-2 毒素の主な損傷であることを知られている。免疫細胞死の分子メカニズムの解明は T-2 毒素による中毒、T-2 毒素及び trichothecenes に関する疾患の予防及び治療の観点から非常に重要である。本研究は、今まで不明な点が多かった T-2 毒素によって誘導される免疫細胞死のシグナル伝達機構の枠組みを明らかにするため、ヒト monocytic leukemia U937 を用いて、種々の細胞死抑制因子を手掛かりとして解析を行った。その結果、T-2 毒素による免疫細胞死シグナルカスケードは、caspase-2 を活性化することにより caspase-8 の活性化、caspase-3 の活性化、核の縮小・断片化、死の順に進行することが解明された。その際、ミトコンドリアは細胞死の amplifier のような役割をしていることが判明した。

以上の知見は、今後の詳細な細胞死分子メカニズム解明へ向けての指針となるばかりでなく、癌の治療薬物の開発に重要な手掛かりを与えうるものであり、学位授与に値するものであると考える。