T-2 toxin initially activates caspase-2 and induces apoptosis in U937 cells

Author(s)  黄, 培新

Citation

Issue Date

Text Version  none

URL  http://hdl.handle.net/11094/47386

DOI

rights

Note

Osaka University Knowledge Archive : OUKA

https://ir.library.osaka-u.ac.jp/repo/ouka/all/

Osaka University
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.

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.

**Summary**

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.