T-2 toxin-induced toxicity in pregnant rats - histopathology and gene expression profiles -

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Summary

T-2 toxin is a trichothecene mycotoxin produced by various species of *Fusarium* spp. Single dose or subacute dose of T-2 toxin induces damage in the lymphoid and hematopoietic tissues, resulting in lymphopenia and immunosuppression in many species. Necrosis/apoptosis is also reported in the gastrointestinal tract and liver. As there are a few reports of T-2 toxin-induced toxicity in pregnant rats, we performed a study to examine T-2 toxin-induced morphological changes in pregnant rats. Single cell necrosis was observed in the thymus, liver, intestines, placenta and fetal liver in pregnant rats treated with T-2 toxin on day 13 of gestation. To investigate gene expression profiles in the liver, placenta and fetal liver in the pregnant rats treated with T-2 toxin, Wistar rats on day 13 of gestation were orally administered with T-2 toxin at a single dosage of 2 mg/kg. Twenty-four hours after treatment, rats were sacrificed. Microarray analysis in the liver, placenta and fetal liver was performed using the Affymetrix Rat Genome U34A chip. The results in these tissues showed the same changes in lipid metabolism-related genes, apoptosis-related genes and oxidative stress-related genes. From these results, the mechanism of T-2 toxin-induced toxicity is speculated that T-2 toxin caused oxidative stress, following the impairment of lipid peroxidation and metabolism-related enzymes. These changes may cause the changes in the intracellular environments, finally resulting in the induction of apoptosis. Further study on the gene expression profiles at the earlier time point should be done to clarify the whole mechanisms of T-2 toxin-induced toxicity.

Key words: T-2 toxin, pregnant rats, histopathology, gene expression

Introduction

T-2 toxin is a trichothecene mycotoxin produced by various species of *Fusarium* spp. T-2 toxin has been found to contaminate foods, animal foods and agricultural

products, and has been reported in many parts of the world¹⁾. Single dose or subacute dose of T-2 toxin induces damage in the lymphoid and hematopoietic tissues, resulting in lymphopenia and immunosuppression in many species. Necrosis/apoptosis is also reported in the gastrointestinal tract and liver²⁻⁴⁾. Furthermore, it is said that pregnant mice treated with T-2 toxin exhibited fetal death and fetotoxicity mainly in the central nervous and skeletal systems⁵⁾. However, mechanisms of fetotoxicity induced by T-2 toxin are still unknown, because there are only a few reports of T-2 toxin-induced toxicity in pregnant rats. Therefore, we first performed the histopathological examination in pregnant rats, placenta and fetal liver⁶⁾.

Molecular approach is necessary to investigate mechanisms of toxic damage induced by chemicals. Recent years DNA Microarray technologies have been developed that allow one to detect the expression of many genes at the same time. To examine the gene expression profiles, we performed microarray analysis in the liver, placenta, and fetal liver in pregnant rats treated with T-2 toxin⁷⁾. We especially focused on genes related to apoptosis, metabolism, and oxidative stress as major mechanisms involved in the T-2 toxin-induced toxicity.

Materials and Methods

Animals Total 18 pregnant Wistar: Slc rats (Japan SLC Co., Ltd., Hamamatsu, Japan) on day 11 of gestation (GD11) were obtained from Japan SLC Co., Ltd., Hamamatsu, Japan. Animals were kept using an isolator caging system (Niki Shoji Co., Ltd., Tokyo, Japan) under controlled conditions (23 ± 2 °C with 55 ± 5 % humidity and a 14 hr light/10 hr dark cycle), and fed commercial pellets (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) and water *ad libitum*.

Treatments Animals were used on GD13. For histopathological study, 6 animals were treated with a single oral dose of 2 mg/kg T-2 toxin (Sigma Chemical Co., St. Louis, MO, USA), and 3 animals were sacrificed by exsanguinations under ether anesthesia at 24 and 48 hours after treatment (HAT), respectively. For microarray study, 3 rats were treated with same way and sacrificed at 24 HAT. In addition, control animals were treated with the vehicle, and were sacrificed in the same way. T-2 toxin was dissolved in corn oil and a dosing volume was adjusted to 2.5 mL/kg.

Pathological examinations After animals were sacrificed, macroscopic examination was performed. Dam's organs and fetuses were weighed. Dam's organs, placentae and fetuses were fixed in 10 % neutral-buffered formalin. Four-mm paraffin sections were stained with hematoxylin and eosin (HE) and subjected to microscopic examination. Cells with fragmented DNA were detected by the TdT mediated dUTP nick end labeling (TUNEL) method using an apoptosis detection kit (Apop Tag, Intergen, Purchase, NY, USA).

RNA extraction and microarray analysis Total RNA was extracted from frozen tissues (up to 0.5 g) from the liver, placenta and fetal liver using the RNeasy Mini Kit (QIAGEN Inc., CA, USA) or TRIzol (Invitrogen, CA, USA). Microarray analysis (total 18 arrays) was performed according to the Affymetrix protocol.

Data analysis The microarray data were analyzed using the Affymetrix software analysis and Spotfire Pro Version 4.2 program (Spotfire Inc., MA, USA). In brief, 4 % trimmed mean normalization was performed in each gene data⁸⁾. Following this, data were compared between the treated group and control group by calculating the Pearson Correlation Value⁹⁾. In the present study, we only analyzed known genes. Finally, we selected genes that gave a value of greater than +1.5 or less than -1.5. The mean value on each gene in the treated group was also statistically compared with that of control by Student's *t*-test or Welch's *t*-test after analysis of homogeneity of variance by F-test.

Results

Body weight, organ weight and mortality In the dams of the T-2 toxin group, although not significant, the body weight showed a tendency to decrease. The weights of the thymus, spleen and liver significantly decreased at 24 HAT. In the fetuses of the T-2 toxin group, a small number of fetal deaths occurred and survival rate of fetuses decreased at 24 HAT, and a decrease in fetal weight was observed.

Histopathological findings Histopathological changes attributable to the T-2 toxin were observed in the lymphoid and hematopoietic tissues, gastrointestinal tracts and genital glands. In the liver, apoptosis and fatty change of hepatocytes and inflammatory cell infiltration were observed (Fig. 1A). In the uterus and placenta, hemorrhage and apoptosis of cytotrophoblasts were observed (Fig. 1B). At 48 HAT, similar changes were observed. In the fetuses of the T-2 toxin group, changes attributable to the treatment were observed in the nervous system, liver, gastrointestinal tracts, and cartilage primordium at 24 HAT. At 48 HAT, sinusoidal dilatation and apoptosis of hematopoietic cells and hepatocytes in the liver were observed (Fig. 1C). There were no histopathological changes in the dams and fetuses in the control group. TUNEL staining demonstrated that apoptosis was induced in the liver, placenta, and fetal liver by T-2 toxin treatment.

Microarray analysis In the present study, we especially focused on genes related to apoptosis, metabolism, and oxidative stress as major mechanisms involved in the T-2 toxin-induced toxicity. In the dam's liver, the expression of 235 genes was induced and the 382 genes were suppressed by T-2 toxin. The expression of cell cycle- and apoptosis-related genes (*p53*, *p21*, and *Bax-a* etc.) was increased. In addition, the expression of oxidative stress-related genes (*Thioredoxin reductase* etc.) was also increased. On the other hand, the expression of lipid metabolism-related genes and drug metabolizing

Fig. 1. Histopathological photographs. A) Liver from a dam treated with T-2 toxin (24 HAT). Fatty change and apoptosis of hepatocytes (arrow) are observed. B) Placenta from a dam treated with T-2 toxin (24 HAT). Apoptosis of cytotrophoblasts (arrow) is observed. C) Fetus liver treated with T-2 toxin (48 HAT). Apoptosis of hematopoietic cells and hepatocytes (arrow) is observed. HE, $\times 165$. (modified from J. Toxicol. Pathology, **16**, 59-65, 2003)

enzymes-related genes (P450s and GSTs) was suppressed. In the placenta, the expression of 187 genes was increased and the expression of 181 genes was suppressed by T-2 toxin. Gene expression of cell cycle- and apoptosis-related genes (*c-fos*, *c-jun*, and *GADD45* etc.) was induced. Gene expression of *Heat shock protein 70* was strongly increased by the treatment. On the other hand, the expression of lipid metabolism-related genes and glutathione S-transferase-related genes was suppressed by T-2 toxin. In the fetal liver, the expression of 15 genes was increased and the expression of 103 genes was suppressed by T-2 toxin. The expression of *heat shock protein 70* (oxidative stress-related gene) was strongly increased. The expression of *Bax-a* was also increased. The expression of metabolism-related genes was generally not induced by T-2 toxin. On the other hand, the expression of lipid metabolism-related genes and GSTs was suppressed. Gene expression of cell cycle- and apoptosis-related genes (*cyclin D*, *c-myc*, and *14-3-3 protein*) was suppressed.

In comparison with the results of 3 tissues (liver, placenta, and fetal liver), changes in lipid metabolism-, apoptosis- and cell cycle-, and oxidative stress-related genes were detected in all tissues. Namely, decreased expression of lipid metabolism-related genes, increased expression of apoptosis- and cell cycle-related genes, and increased expression of oxidative stress-related genes were detected (Fig. 2).

Fig. 2. Summary of microarray analysis in the dam liver, placenta and fetal liver.

Discussion

Pregnant Wistar rats on GD13 were treated with a single oral dose of 2 mg/kg T-2 toxin, and were sacrificed at 24 and 48 HAT, and subjected to histopathological examination. It is reported that T-2 toxin induces apoptosis in the lymphoid tissues and intestine²⁻⁴). In our study, apoptotic changes were observed in the lymphoid tissues including the thymus and spleen, intestine, and fetal brain⁶). It has been considered that fetotoxicity of T-2 toxin is secondary to maternal toxicity. T-2 toxin easily passes the placenta and distributes to fetal tissues¹⁰), and the changes observed in fetuses were essentially the same as those observed in dams in the present study. Judging from those findings, fetotoxicity is not secondary to maternal toxicity but a direct toxicity to fetuses. Therefore, T-2 toxin-induced changes may be caused by the same mechanisms in these tissues.

From the results of microarray analysis, the expression of apoptosis/cell cycle-related genes, lipid metabolism-related genes, drug metabolizing enzyme genes and oxidative stress-related genes was detected in the liver, placenta and fetal liver. T-2 toxin enhances lipid peroxidation in the liver¹¹). Oxidative stress causes lipid peroxidation and induces mitochondrial dysfunction. This mitochondrial dysfunction causes fatty acid β -oxidation and induces fatty liver¹²). In our study, fatty liver was observed in the liver, suggesting that the fatty change in the liver may be related with oxidative stress caused by T-2 toxin.

Increased apoptosis in the liver, placenta and fetal liver was observed histopathologically in the present study, and microarray analysis also showed the

increased expression of apoptosis-related genes. At the same time, the expression of cell cycle accelerating genes was also induced by T-2 toxin. Furthermore, the expression of oxidative stress-related genes and repressor of apoptosis was induced in the placenta and fetal liver. This suggests that T-2 toxin induced not only apoptosis but also cell repair and proliferation at 24 HAT.

In our study, the expression of drug-metabolizing enzyme genes was suppressed or showed the tendency of suppression by T-2 toxin treatment in the liver, placenta and fetal liver. This result was consistent with those of reported study¹³⁾. It is reported that lipid peroxidation caused by oxidative stress might affect the P450 content in the liver¹⁴⁾. Therefore, oxidative stress might be an effective factor of P450 suppression.

In conclusion, histopathological study showed that T-2 toxin may induce the apoptotic changes of the same nature in the liver, placenta, and fetal tissues. Microarray analysis in the liver, placenta and fetal liver showed the same changes in lipid metabolism-related genes, drug metabolizing enzyme genes, apoptosis-related genes and oxidative stress-related genes. From these results, the mechanism of T-2 toxin-induced toxicity is speculated that T-2 toxin causes oxidative stress, following the impairment of lipid peroxidation and metabolism-related enzymes. These changes may cause the changes in the intracellular environments, finally resulting in the induction of apoptosis. Further study on the gene expression profiles at the earlier time point should be done to clarify the whole mechanisms of T-2 toxin-induced toxicity.

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