Bull. Environ. Contam. Toxicol. (1987) 39:299–303

Environmental Contamination and Toxicology

Teratogenicity of Oral Chaetochromin, a Polyphenolic Mycotoxin Produced by *Chaetomium* spp., to Mice Embryo

Yoshitake Ito[1,*] and Kohichiro Ohtsubo[2]

[1]Institute for Medical Science of Aging, Aichi Medical University, Aichi-gun, Aichi 480-11, Japan and [2]Department of Clinical Pathology, Tokyo Metropolitan Institute of Gerontology, Itabashi-ku, Tokyo 173, Japan

Chaetochromin (Sekita et al. 1980), bis(naphthodihydropyran-4-one), is a recently isolated polyphenolic mycotoxin produced by several species of Chaetomium (Udagawa et al. 1979; Udagawa 1980). A screening test of the toxin on HeLa cells in culture reveals a potent cytotoxicity (Sekita et al. 1981). According to our recent studies (Ohtsubo 1980; Ohtsubo 1982), the toxin induces delayed liver injuries, bone marrow aplasia and atrophy of lymphatic tissues in mice. Feeding of the moldy rice of C. gracile which produces only chaetochromin also leads to the same changes at concentration of 30 ppm (Ohtsubo 1982). But with lower dosages of 10 ppm, no noticeable pathological or hematological changes were observed after one year of feeding (unpublished result). Since many mycotoxins cause embryotoxic and teratogenic effect (Hayes 1981), we examined the effects of this toxin on the pregnant mice and their embryos.

MATERIALS AND METHODS

Moldy rice was prepared and supplied by Drs. H. Kurata and F. Sakabe of National Institute of Hygenic Sciences. The moldy rice containing 2900 ppm chaetochromin (assayed by Dr. S. Sekita, National Institute of Hygenic Sciences) was mixed in the standard animal feed (CRF-1, Oriental Yeast Co. Ltd., Tokyo) to prepare pellets. The final concentrations of the toxin in the pellet were adjusted to 30 and 10 ppm.

Male and female mice of ICR strain were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu, Japan). Animals were housed in plastic cages (4 to 5 mice/cage in preexperimental period and during pregnancy, 1 male and 2 females while mating) in a room designed to control temperature at 23±1°C, relative humidity at 55±5% and

*Correspondence and reprint requests.

12 hr light cycle. Laboratory animal chow (CRF-1) and tap water were available ad libitum. The virgin females aged 8 weeks were paired overnight with males. The day on which a vaginal plug or sperms in the vaginal smears were detected was designated as day 0 of gestation. Fertilized females were randomly assigned to one of the four dose groups or control group (Table 1).

Pregnant mice were given experimental diet from day 0 to sacrifice on day 18, or from day 7 to 9 or from day 10 to 12 of gestation. Dams were weighed every morning and sacrificed by cervical dislocation on day 18 of gestation. On exposing the uterus, number of total implantations, resorptions and live or dead fetuses was recorded. Live fetuses were inspected for external malformations and then weighed. After fixation with 10% formalin or 95% ethanol, they were evenly divided into two groups for visceral observation by razor sectioning and for skeletal observation by staining with Alizarin red S. One or 2 fetuses from each litter were examined histologically. Maternal organs were also weighed and examined histologically.

RESULTS AND DISCUSSION

Fed with diet containing chaetochromin at 10 or 30 ppm throughout the course of pregnancy, the body weights of the fetuses were significantly reduced ($p<0.05$ and $p<0.01$, respectively, by Student's t-test). In the group of 0-18 day feeding at the higher dose, there was a significant ($p<0.001$, chi square-test) increase of late resorptions (fetal death after the formation of the placenta) and a significant ($p<0.005$, chi square-test) decrease in the number of living fetuses. No increase in death or resorption rate in the early stage was observed. However, chaetochromin induced malformation in the fetuses of this group.

As the major malformation, exencephaly was observed in 10 out of 97 living fetuses from the dams given diet containing 30 ppm chaetochromin for 18 days. Two of 10 anomalous fetuses had open eye lids and the other 2 had agnathia. The occurrence of exencephaly was of significantly high rate by Fischer's direct probability test ($p<0.005$). All fetuses with exencephaly had excess, blood-tinged, amniotic fluid. In the coronal plane by the razor sectioning, the diencephalon was protruded over the telencephalon. The roof of the fourth ventricle was connected with the skin of the temporal area. The hemispheres enclosed the lateral ventricles while the choroid plexuses were displaced dorsally or laterally. Histologically, the capillaries were proliferated scatteringly in the protruded brain

Table 1. Effects of chaetochromin on the mouse fetuses

Concentration in the feed (ppm)	Feeding periods (days)	Total implants / No. of dams	No. of living (%)	No. of resorbed early (%)	No. of resorbed late (%)	No. of died (%)	Body weight M±SD (g)
0		116 / 10	107 (92.2)	8 (6.9)	1 (0.9)	0 (0.0)	1.38±0.14
10	0 - 18	79 / 6	74 (93.7)	4 (5.1)	0 (0.0)	1 (1.3)	1.32±0.12*
30	0 - 18	126 / 10	97*** (77.0)	7 (5.6)	20**** (15.9)	2 (1.6)	1.28±0.15**
30	7 - 9	71 / 5	64 (90.1)	4 (5.6)	2 (2.8)	1 (1.4)	1.39±0.11
30	10 - 12	82 / 6	75 (91.5)	5 (6.1)	1 (1.2)	1 (1.2)	1.37±0.12

*,**,***,****, significantly different (* $p<0.05$, ** $p<0.01$, *** $p<0.005$, **** $p<0.001$) from untreated control group.

accompanied with degeneration and hemorrhage. In the skeletal observation, the fetuses with exencephaly were defective of the frontal, parietal, interparietal and supraoccipital bones. In the case of exencephaly with agnathia, the mandibula was defective in addition to the above described skull defects.

In both groups of short term feeding with the higher dose, no major malformation was found. As a minor variation, however, the lumbar ribs were increased in the 7-9 day feeding group (14.3%) and the 10-12 day group (13.0%), compared with the control group (4.7%).

There was no significant difference between control and experimental animals in the pattern of maternal weight gain during pregnancy, the organ weights and histological findings. Histological examination of the dams revealed a slight to moderate increase of mitosis in the liver cells of the animals in all experimental groups.

Thus, presently tested mycotoxin, chaetochromin, induced exencephaly in about 10% of the fetuses by feeding diet containing 30 ppm of the toxin from 0 to 18 pregnant days. The critical period for the development of exencephaly, a relatively common anomaly induced by many chemicals, agnathia and open eye lids is the day 7-8 of pregnancy (Murakami et al. 1968). In the present study, however, malformations and late resorptions were not induced in the group provided for a short-term including the critical period, probably due to the slow-acting nature of chaetochromin (Ohtsubo 1982). This was also reflected in the fact that incidence of fetal absorption was more frequent in the later stage even by continuous feeding. Further investigations are necessary to clarify the more direct effects of the toxin when administered in the critical period.

In the DDD strain (An inbred strain of albino mouse established at the Institute of Medical Science, the University of Tokyo) of mice fed 30 ppm chaetochromin, the body weight loss was marked in the first week of administration. The typical hepatic lesion and bone marrow damage developed in the second week and later. In this stage leukopenia and decreased number of nucleated cells in the bone marrow were marked (Ohtsubo 1982). Even with the high dose of 200mg/kg b.w. of purified chaetochromin, administered by gavage in DMSO, the DDD strain of mice were unaffected until third day, when low activity, loss of glossiness of the hair and anorexia appeared and the body weight fell rapidly thereafter (Ohtsubo 1980). Consequently, divided doses of 30 ppm, or 4mg/kg b.w./day for consecutive 7 or 8

days (upto the day of critical period for exencephaly) seemed to have been necessary to induce fetal damage. It is noticeable that the histological findings of maternal organs are not so severe as reported previously in nonpregnant animals, possibly because of the difference of the mouse strain. Additionally, degradation of the toxin during feed preservation for about a year has been minimum by chemical analysis (Dr. S. Natori, personal communication).

REFERENCES

Hayes AW (1981) Mycotoxin teratogenicity and mutagenicity. CRC Press Inc., New York

Murakami U, Suzuki M, Baba K (1968) Prenatal medicine. Igaku Shoin, Tokyo

Ohtsubo K (1980) Oral toxicity of chaetochromin, a new mycotoxin produced by Chaetomium virescens, to mice. Proc Jpn Asso Mycotoxicol 12:28-29.

Ohtsubo K (1982) Hematopoietic injury and liver necrosis in the mice fed chaetochromin-containing moldy rice diet. Proc Jpn Asso Mycotoxicol 15:25-27.

Sekita S, Yoshihira K, Natori S (1980) Chaetochromin, a bis(naphthodihydropyran-4-one) mycotoxin from Chaetomium thielavioideum: application of ^{13}C-^{1}H long-range coupling to the structure elucidation. Chem Pharm Bull 28:2428-2435.

Sekita S, Yoshihira K, Natori S, Udagawa S, Mouri T, Sugiyama Y, Kurata H, Umeda M (1981) Mycotoxin production by Chaetomium spp. and related fungi. Can J Microbiol 27:766-772.

Udagawa S (1980) New or noteworthy Ascomycetes from Southeast Asian soil. I. Trans Mycol Jpn 21:17-34.

Udagawa S, Mouri T, Kurata H, Sekita S, Yoshihira K, Natori S, Umeda M (1979) The production of chaetoglobosins, sterigmatocystin, O-methylsterigmatocystin, and chaetocin by Chaetomium spp. and related fungi. Can J Microbiol 25:170-177.

Received March 9, 1987; accepted April 15, 1987.