STERIGMATOCYSTIN INDUCED ADENOCARCINOMA OF THE LUNG AND ATYPICAL HYPERPLASIA OF GLANDULAR STOMACH IN MICE

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Aspergillus versicolor was isolated from the gastric juice of patients with chronic stomach diseases in high-risk area of gastric cancer. Mice fed with Aspergillus-inoculated corn flour developed adenocarcinoma of the lung in 15 of 35 mice (42.9%) and atypical hyperplasia of the glandular stomach in 15 of 35 mice (37.4%). Sterigmatocystin was identified by high performance liquid chromatography (HPLC) and fluorescence spectrophotometry in the extract of Aspergillus-inoculated corn flour. The results suggest that the mycotoxin sterigmatocystin may play a potential role in carcinogenesis in human.

The relevance of Asperillus versicolor to genesis of stomach cancer was reported in our country after epidemiological studies, but the similar investigation by experiment is lacking. To detect the carcinogenicity of Aspergillus versicolor for stomach, the carcinoma induction was made in mice by feeding the corn flour inoculated with Aspergillus versicolor, which was isolated from the gastric juice of patients with chronic stomach diseases in high-risk area of stomach cancer and the sterigmatocystin was also identified in the extract of the Aspergillus-inoculated corn flour.

MATERIALS AND METHODS

Analysis of Sterigmatocystin

The Culture of Aspergillus Versicolor

Aspergillus versicolor, which was isolated from the gastric juice of patients with chronic stomach diseases in high-risk area of gastric cancer, was inoculated in sterilized corn flour and incubated for 14 days at 25°C. After taking some of the Aspergillus-inoculated corn flour as sample for determining no other fungi growing, the Aspergillus versicolor was inactivated at 100°C for 30 minutes. Meanwhile the sterilized corn flour, in which the Aspergillus versicolor was not inoculated, was as control.

Extraction of Sterigmatocystin

Fifty grams Aspergillus-inoculated corn flour was extracted three times with chloroform. The supernatant liquid was filtrated and concentrated to 5 ml by evaporation at 37°C. The 2 ml concentrated solution was taken in a tube and volatilized up at 70°C. The nitrogen was connected to the tube for driving out the volatile substance by blowing nitrogen. 2 ml solution of methyl alcohol and water (3:2) was added and cleaned up with n-hexane. The remaining water phase was served as sample for analysis of sterigmatocystin. The control corn flour (50 g) was extracted and control sample was made with the same method as above.

HPLC Analysis

1. Analytic condition: Stationary phase was bonded reversed phase Nucleosil C18. The column
dimension was 120×46 mm. The column pressure was 9.7 MPa, and column temperature 14.5°C. The solution of methyl alcohol and water (3:2) was used as mobile phase. The flow rate was 0.8 ml/min. 2. Detecting condition: The excitation was at 380 nm and emission at 426 nm. The full recorder range was 10 mV. 3. Instrument: Series 3 Liquid Chromatograph and MPF-44B fluorescence spectrophotometer (Perkin-Elmer Co. USA).

The retention time was used for determination of quality and the peak height in chromatogram for determination of quantity of the mycotoxin.

**Induction of Cancer in Mice**

**Diet Preparation**

The *Aspergillus*-inoculated corn flour was mixed with commercial diet of mice and the pellet diet was made. The proportion of the *Aspergillus*-inoculated corn flour in the pellet diet was 1/3 (first diet) and 1/2 (second diet) respectively. The control corn flour was used for making control diet in the same process.

**Examination**

The animals were killed in two batches at the 58th and the 78th week of the experiment and autopsied. All organs were examined carefully. The stomach, esophagus, lung, liver and other suspicious organs observed grossly were removed, fixed in 10% formalin and embedded in paraffin. Sections were routinely stained with Hematoxylin and Eosin.

**Animal and Groups**

The NIH mice, purchased from Center of Experimental Animal, the Academy of Medical Sciences of China, weighing 16—19 g, were divided into test and control groups randomly, 40 mice of both sexes each group. Housed in plastic cages (5 per cages) with metal tops, the mice were kept under standard conditions. The mice in the test group were fed with pellet diet containing *Aspergillus*-inoculated corn flour, first diet for 47 weeks, then the second diet for another 23 weeks, and then were given commercial diet. The mice in the control group were fed with control diet and the treatments were the same as that in the test group. The mice in both groups drank tap water ad libitum. The animal were inspected everyday, including weekends and holidays and weighed every two weeks.

**RESULTS**

**Analysis of Sterigmatocystin**

It was found with HPLC assay that there was sterigmatocystin 0.117 mg per Kg *Aspergillus*-inoculated corn flour. Otherwise there was no mycotoxin in the corn flour which served as control.

**Induction of Carcinoma**

The general conditions of animals in the test group were much poorer than that of mice in the control group. The body weight of the mice in the test group decreased at the later stage of the experiment. 5 mice naturally died at the early stage and pneumonia and punctate hemorrhage in lungs was observed. The number of effective animal was 35 and their major lesions were as following:

**Lesions in Lung**

The 21 mice were killed and autopsied at the 58th week of the experiment. Among them 7 mice were found to have nodules in lungs, most of which were multiple. The nodules were greyish white, solid, and from cap of pin to milium in size. Under microscope all of them were adenocarcinoma (Figure 1). Meanwhile atypical hyperplasia occurred in bronchiols of 4 mice. The remaining 14 mice were killed at the 78th week of experiment. Adenocarcinoma and atypical hyperplasia were found in 8 and 3 mice of them. The histological architecture of the lesions were similar in mice of the 58th and the 78th week.