CARCINOGENICITY OF FUSARIN C ISOLATED FROM 
FUSARIUM MONILIFORME

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Fusarium moniliforme, a fungus of established carcinogenic potential, is one of the most common fungal contaminants of maize, millet and other grains in Linxian County, China. Fusarin C, a major product of F. moniliforme grown on corn in the laboratory, is mutagenic in Salmonella tester strains and in V<sub>79</sub> cells. Fusarin C showed several characteristics of malignant transformation including the implantation of the rat esophageal epithelial cell line (RE — 525) in nude mice. The present work demonstrated that fusarin C can induce esophageal and forestomach carcinomas in DBA mice and Wistar rats, and thus the experimental results substantiated further the carcinogenicity of fusarin C.

Fusarin C (FC) is a mycotoxin isolated from one of the most common fungi, Fusarium moniliforme Sheldon, which grows on maize, millet and other grains in China, Southern Africa and North America. Based upon the distribution of F. moniliforme and the consumption of corn contaminated with this fungus, an association with human esophageal cancer has been suggested, especially in the areas of Linxian County, China and Transkei, Southern Africa. In our previous studies, it was shown that papillomas and squamous-cell carcinomas of the esophagus and forestomach can be induced in rats fed a corn meal culture of this fungus. During the past decade several compounds produced by this fungus have been identified, some of which are toxic and mutagenic. The positive results of mutagenicity tests have been reported previously. FC (Figure 1) is capable of inducing sister chromatid exchanges (SCE), micronuclei, chromosomal aberrations, and 6-thioguanine-resistant mutants in V<sub>79</sub> cells. Furthermore, FC increases the number of DNA breaks in bacteria in the presence of a metabolic activation system, induces asynchronous replication of the polyoma DNA sequences in H3 rat fibroblast cells, and enhances the carcinogenicity of alkylates 4-(p-nitrobenzyl)pyridine in the absence of an added metabolic activation system.

Recently, DNA-binding of 3H-FC was observed in explants of rat esophagus. FC treatment of an esophageal epithelial cell line (RE-525 produced the following indications of malignant transformation:

![Fig. 1. Structure of Fusarin C.](image)
1. Colonies were formed after seeding transformed cells both into selective medium free of epidermal growth factor, serum and on semisolid agar.

2. There was an increase in chromosome number and chromosomal aberrations.

3. The expressions of N-ras, c-myc and V-erb-B oncogenes were enhanced in these cells.

4. The transformed cells lost response to TPA (12-0-tetradecanoyl-phorbol-13-acetate) in modulating c-myc expression.

5. Squamous-cell carcinomas developed after inoculating these FC-treated RE-525 cells subcutaneously into BALB/c nude mice.

Recently, Jaskiewicz, et al. have postulated that FC may not be the mycotoxin responsible for the development of esophageal cancer in rats fed a diet containing culture material of *F. moniliforme*.

To determine the carcinogenic activity of FC and non-purified FC substances, we intubated mice chronically with thinlayer chromatography (TLC) purified FC and treated rats similarly with the methanol extract of corn meal inoculated with *F. moniliforme*. Tumor development was observed in the esophagus and forestomach.

**MATERIALS AND METHODS**

FC was isolated and identified as previously described, and it is a yellow, noncrystallized compound (C₃₃H₃₉N O₇). Crude FC was obtained by silica gel column chromatography (elution with chloroform/methanol, 9:1) of methanol extract of *F. moniliforme*, strain 07, cultured in corn; and purification of FC was carried out by TLC (silica gel chloroform/methanol, 9:1, Rf 0.44). Purified FC was further confirmed by nuclear-magnetic resonance (NMR) spectrum (JEOL, FX-90Q), and the data were comparable with that reported by W.C.A. Gelderblom, et al. The purities of TLC-purified FC and nonpurified FC were 86% and 19.5%, respectively, as detected by high performance liquid chromatography (HPLC). FC was stored in the dark at −20°C.

Female mice and rats were used in the present experiments. Forty DBA mice, aged 30—35 days, weighing 18—25 g were administered by gavaging twice weekly 0.5—0.05 mg/ml TLC-purified FC, and since FC was toxic to experimental animals the dosages given were decreased gradually. FC was dissolved first in a minute amount of ethanol and then diluted with distilled water just before used. Twenty DBA mice served as untreated controls. Forty Wistar rats, about 40 days old, weighing 80—120 g were used, and 2 mg/ml nonpurified FC was intubated twice weekly and later increased to 3 mg/ml nonpurified FC in each dose when their body weights were 300 g or over. Doses administered are indicated in Tables 1 and 2. A group of 25 female Wistar rats served as untreated controls. At the end of experiments, the esophagus, forestomach, glandular stomach, liver, lungs and other organs were examined and preserved for histopathogical study.

**Table 1. Fusarin C induced esophageo-forestomach carcinoma in DBA mice**

<table>
<thead>
<tr>
<th>Time in days (FC* total dose)</th>
<th>No. of mice</th>
<th>Hyperplasia</th>
<th>Dysplasia</th>
<th>Papilloma</th>
<th>Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>9—27 (1.5—3mg)</td>
<td>14</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>122—150 (3.25—5mg)</td>
<td>9</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>292 (10.1mg)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>469—502 (8.2—12.6mg)</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>655 (14.2mg)</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>18</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

* TLC-purified FC