Toxic Fungi isolated from Uganda Foodstuffs: Chronic toxicity of fungal culture filtrates

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Abstract : For the detection of chronic toxicity of possible unknown mycotoxins in culture filtrates of fungi which had been isolated from Uganda foodstuffs, long-term animal experiments were carried out employing 4- to 6-week-old C3H/HeJ male mice by the method of intraperitoneal injection. Out of 209 strains of fungi which showed strong acute toxicity on mice in the previous short-term experiments, 12 strains of *Aspergillus* (*Asp.*) and two strains of *Penicillium* (*Pen.*) were selected for the study. Two strains of *Asp. flavus* ; GN−22−1−5 and GN−25−1, *Asp.* oryzae GN−21−2−4 and *Asp. candidus* SM−10−2 caused atrophy of the liver. However, no obvious fibrosis, cirrhosis or other chronic lesions of the liver were observed. *Asp. flavus* BE−13−4 was the only strain that caused marked pleomorphism of the liver cell nuclei. A few strains of *Aspergillus* and *Pen. funiculosum* GN−48−8 caused swelling and nuclear pleomorphism of proximal tubules of the kidney. Marked hypospermatogenesis of the testis was seen in mice treated with *Pen. funiculosum* GN−48−8. The methods for administraion of fungal culture filtrates were discussed.

Mycotoxin, toxic metabolites of fungi, in foodstuffs have been postulated to be one of the possible environmental factors in the etiology of diseases of the liver and

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other organs in human beings as well as domestic animals, especially in the tropics.

Isolation of fungi from African food samples, which were collected from different parts of Uganda in East Africa (Alpert *et al.*, 1971), and determination of fungal metabolites; Aflatoxins, kojic acid, β-nitrosopropionic acid and nitrosocompounds in fungal culture filtrates were performed (Kawasaki *et al.*, 1971). Bacteriocidal effects and λ-bacteriophage inductions of fungal culture filtrates were also studied. Furthermore, a survey of acute toxicity of fungal culture filtates using mice was made (Itakura and Kinosita, 1975).

The present paper is limited to reporting the results of the long-term animal experiments for detection of chronic toxic effects of fungal culture filtrates.

MATERIALS AND METHODS

The places and methods of collection of food samples in Uganda have been reported by Alpert *et al.* (1971). The food samples tested and methods of preparation of fungal culture filtrates using glucose ammonium nitrate medium (B medium) and mycological broth medium (M medium) are shown in the preceding reports (Kinosita *et al.*, 1974).

From the results of the previous short-term animal experiments (Itakura and Kinoshita, 1975), 14 strains of fungi which showed positive acute toxicities were selected. The experiments were carried out employing 4- to 6-week-old C3H / HeJ male mice fed with 8% low protein diet. For testing strains of fungi and methods of administraion of materials, these studies were divided into two experiments as follows:

Experiment I:

The strains of fungi were *Asp. petrakii* GN-4-3 (Exp. 151) and *Asp. ostianus* BE-3-1 (Exp. 152). Both of them were cultured on B medium.

As a method for administration, 0.5 ml of the culture filtrate of each fungal strain adjusted to pH 5.0-5.5 was daily injected intraperitoneally in a mouse, 31 mice per each fungal strain, ten times in all at the beginning of the experiment. Thereafter no treatment was performed. On the 330th day after the first injection the mice were sacrificed for pathological observation.

Experiment II:

The M culture preparations of the following 12 strains of testing fungi; *Asp. oryzae* GN-21-2-4 (Exp. 435), *Asp. fumigatus var. ellipticus* GN-21-5-1 (Exp. 436), *Asp. flavus* GN-22-1-5 (Exp. 437), *Asp. flavus* GN-25-1 (Exp. 438), *Asp. ostianus* GN-35-1 (Exp. 439), *Asp. ficuum* GN-39-2 (Exp. 440), *Asp. ostianus* PE-11-1-5 (Exp. 441), *Asp. flavus* BE-13-4 (Exp. 442), *Asp. candidus* SM-10-2 (Exp. 443), *Asp. ficuum* SM-10-6 (Exp. 444), *Pen. funiculosum* GN-27-4-2 (Exp. 445) and *Pen. funiculosum* GN-48-8 (Exp. 446) were diluted with dist. water to half concentration and were

adjusted to pH 5.0-5.5.

As a method for administration, 0.5ml of the diluted culture filtrate of each fungal strain was daily injected intraperitoneally in a mouse, usually 20 mice per each fungal strain, ten times in all at the beginning of the experiment. During the last eight weeks of the experiment, five days per week, five mice out of each 20 mice were administered 10% culture filtrate in drinking water.

Two groups of mice were used for controls, one was composed of ten mice treated with M culture medium only and the other was composed of 25 mice without any treatment except feeding with low protein diet.

All mice were sacrificed about 170 days after the first injection.

RESULTS AND DISCUSSION

Summarized results of pathological studies are given in Table.

Experiment I:

Out of 31 mice which were treated with *Asp. petrakii* GN−4−3, 11 mice were sacrificed on the last experiment day and showed moderate swelling of the liver macroscopically. This fungal strain caused moderate liver lesions in the short-term experiments and showed bacteriocidal effects on *Bacillus megatherium*.

Out of 31 mice being treated with *Asp. ostianus* BE−3−1, 16 mice were sacrificed on the last experiment day. Although this fungal strain showed acute toxicity on the liver and moderate bacteriocidal effects on *Bacillus megatherium*, no remarkable change was recognized in the long-term experiments.

Experiment II:

A few mice per each fungal strain were killed before the day of sacrifice by non-specific infection and other accidents. All mice were examined macroscopically and one mouse per each five mice which were treated with the culture filtrate in drinking water was examined microscopically.

Atrophy of the liver without any fibrosis was seen in mice treated with *Asp. oryzae* GN−21−2−4, *Asp. flavus* GN−22−1−5, *Asp. flavus* GN−25−1 and *Asp. candidus* SM−10−2. Although these strains of fungi caused peritonitis followed by loss of body weight and atrophy of the liver in the previous short-term experiments, no obvious fibrosis, cirrhosis or other chronic lesions of the liver were observed in the long-term experiment. *Asp ostianus* GN−35−1 caused adhesive peritonitis and slight swelling of the liver. Microscopically, moderate degree of pleomorphism of liver cell nuclei was seen. *Asp. flavus* BE−13−4 is the only strain that caused marked pleomorphism of liver cell nuclei in this study.

Asp. oryzae GN−21−2−4, *Asp. fumigatus var. ellipticus* GN−21−5−1, *Asp. ostianus* PE−11−1−5 and *Pen. funiculosum* GN−48−8 caused acute lesions of the kidney, but they showed only slight to moderate swelling or nuclear pleomorphism of renal

Table. Pathological effects of fungal culture filtrates on mice

Experiment number	Strain of fungus		Culture medium	Autopsy findings
151	GN-4-3	Asp. petrakii	B	Liver : moderate swelling
152	BE-3-1	Asp. ostianus	B	Liver : slightly yellowish
435	GN-21-2-4	Asp. oryzae	M	Liver : atrophy Kidney : moderate swelling and nuclear pleomorphism of proximal tubular epitherium
436	GN-21-5-1	Asp. fumigatus var. ellipticus	M	Kidney : slight nuclear pleomorphism of proximal tubular epitherium
437	GN-22-1-5	Asp. flavus	M	Liver : atrophy Testis : slight hypospermatogenesis
438	GN-25-1	Asp. flavus	M	Liver : atrophy
439	GN-35-1	Asp. ostianus	M	Adhesive peritonitis Liver : slight swelling, moderate nuclear pleomorphism of liver cells
440	GN-39-2	Asp. ficuum	M	Liver : moderate pleomorphism of nuclei
441	PE-11-1-5	Asp. ostianus	M	Thickening of renal capsule (Perinephritis?) Kindney : moderate swelling of proximal tubular epitherium Testis : s ight hypospermatogenesis
442	BE-13-4	Asp. flavus	M	Liver : marked pleomorphism of nuclei
443	SM-10-2	Asp. candidus	M	Liver : atrophy Kidney : degeneration of proximal tubular epitherium
444	SM-10-6	Asp. ficuum	M	Liver : moderate pleomorphism of nuclei Testis : slight hypospermatogenesis
445	GN-27-4-2	Pen. funiculosum	M	no remarkable change
446	GN-48-8	Pen. funiculosum	M	Liver : moderate pleomorphism of nuclei Kidney : moderate swelling of proximal tubular epitherium Testis : marked atrophy and hyposperma-togenesis
447	Control (M-medium)			Liver : moderate pleomorphism of nuclei Kidney : slight swelling of proximal tubular epitherium
448	Control (No treatment)			Liver : moderate pleomorphism of nuclei

tubular epithelium in the long-term experiments. Degeneration of proximal tubular epithelium of the kidney was noted by *Asp. candidus* SM−10−2. In the case of *Pen. funiculosum* GN−48−8, marked atrophy of the testis was observed macroscopically and hypospermatogenesis microscopically. On the contrary, no acute pathological effects on the testis was found in the short-term study.

In the control group treated with M culture medium alone, moderate pleomorphism

of liver cell nuclei and slight swelling of proximal tubular epithelium of the kidney could be recognized.

The M culture preparations of fungi which were selected for this long-term study showed strong acute toxicities on mice in the previous short-term experiments. It was necessary, therefore, to dilute the crude culture filtrates with dist. water in order to carry out the long-term experiments successfully. This experiment was designed to obtain chronic pathological effects on mice by possible mycotoxins in the culture filtrates. From the results of the short-term experiments, it was estimated that the target organ of mycotoxins of *Asp. fumigatus var. ellipticus* GN−21−5−1, *Asp. ostianus* GN−35−1 and *Pen. funiculosum* GN−27−4−2 was the liver and that of *Asp. ficuum* GN−39−2 was the kidney. However, no remarkable chronic lesion or tumorous change in any organ that was expected to be obtained could be seen in this study. It appears that the toxic amount administered to the mice was too small to produce chronic effects on mice. Moreover, it seems that the length of the administration of culture filtrates was not long enough to produce chronic changes in mice.

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東アフリカ・ウガンダ国の主要食品類より分離された毒性真菌類: 真菌培養濾液の慢性毒性

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肝疾患が多発する東アフリカにおいて食品寄生真菌類の毒性代謝産物（マイコトキシン）の産生について検索した. ウガンダ国のほぼ全域に亘って日常主要食品類（落花生, 米, 豆類など）を

収集した．そして各種真菌類を分離培養して，その培養濾液をマウス（C3H/HeJ）に腹腔内投与することによって長期間（170日～330日）に生じる臓器障害を病理学的に調べた．既に行った短期間の実験で毒性を示した 209 strains の真菌類の中からとくに強い毒性を示した *Aspergillus* と *Penicillium* のそれぞれ 12 strains および 2 strains とを選んで試験した．*Aspergillus* の 5 strains が肝臓の萎縮や肝細胞核の大小不同などを生ぜしめたが，肝線維症や肝硬変などの明らかな慢性障害像はみられなかった．*Penicillium* の1 strain が睾丸の萎縮を生ぜしめた．そのほか二，三の *Aspergillus* や *Penicillium* が腎尿細管の上皮細胞の腫大や核の大小不同を生ぜしめたが著しい慢性障害像ではなかった．本実験で強い慢性障害像を得られなかったことは，検索した真菌の毒性が既に前実験で確認されているので，マウスへの投与量および投与期間が十分ではなかった為と考えられる．

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