ANTIVIRAL ACTIVITY OF TRICHOTHECIN

Sir :

In our antianimal-virus antibiotic screening using paper-disc agar diffusion technique[1], the mycelial acetone extract of *Trichothecium roseum* LINK IFO 5772 showed remarkable antiviral activity against Newcastle disease virus strain Miyadera (NDV) infected on chick embryo fibroblast monolayer (CEF). The extract also showed growth-inhibitory activity against *Candida albicans* strain Ch. The antifungal activity seemed to parallel antiviral activity and the two activities had the same Rf values in the bioautograms of the extract using *C. albicans* and NDV as indicators when the extract was developed on thin-layer chromatography. Therefore, an agar diffusion assay with *C. albicans* was developed to speed up fractionation and isolation. The active principle was obtained in crystalline form from the mycelial extract through silica gel column chromatography.

Table 1. Inhibition of cytopathic effect in NDV infected HeLa cells by trichothecin

Trichothecin (mcg/ml)	Cytopathic effect		
	2 days	3 days	5 days
0.250	− − −	− − −	− − −
0.125	± − −	± ++ ++	+++ +++ +++
0.063	+ + +	++ ++ ++	+++ +++ +++
0.032	+ + +	++ ++ ++	+++ +++ +++
Control	+ + +	+++ +++ +++	+++ +++ +++

Inhibition test in tube culture were performed as follows : HeLa cells were cultured for 48 hours at 37°C at an initial concentration of 3.0×10^5 cells per 0.5 ml of EARLE's solution supplemented with 15 % of calf serum. After 48 hours the medium was removed and 0.5 ml of ND virus suspension diluted with EARLE's solution was added to the washed cell monolayer. After incubation for 2 hours at 37°C the cells were again washed and the maintenance media containing various amounts of trichothecin, with calf serum reduced from 15 % to 2 %, was added to the cell monolayers. A control without trichothecin was included in each test. Inhibition of cytopathic effect (CPE) was determined by microscopic observation. Triplicate tubes were used in each concentration. Criteria for CPE ; − no CPE, + 1~25 % of the cells showed CPE, ++ 26~50 % of the cells showed CPE, +++ 51~75 % of the cells showed CPE, and ++++ 76~100 % of the cells showed CPE.

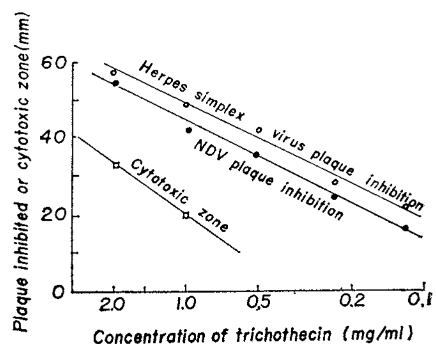
Ultraviolet absorption maxima, melting point and microelementary analysis were virtually identical with those of trichothecin reported by FREEMAN and MORRISON[2]. The infrared absorption spectrum was also identical with that of authentic trichothecin. In this communication, the antiviral activity of trichothecin is described.

Trichothecin, which was discovered by FREEMAN and MORRISON, is an antifungal antibiotic[3]. Trichothecin contains a sesquiterpene alcohol, trichothecolone, esterified with isocrotonic acid[4]. The sesqui-terpene moiety is confirmed to be derived from 3 molecules of mevalonic acid[5]. It was found that trichothecin was effective against some plant viruses[6].

Fig. 1 demonstrates the dose-response relationship in agar-diffusion plaque-inhibition

Fig. 1. Herpes simples and ND viruses plaque inhibition by trichothecin.

Primary chick embryo fibroblasts were grown at an initial concentration of 4.0×10^7 cells per 15 ml of EARLE's solution supplemented with 0.5 % of lactalbumin hydrolyzate, 0.1 % of yeast extract and 5 % of calf serum in a glass petri dish, 9 cm in diameter. The cultures were incubated for 48 hours at 37°C under 5 % CO_2-containing atmosphere. Then the medium was removed and the monolayer sheet was covered with 1.5 ml of virus suspension diluted with EARLE's solution. After incubation for 2 hours at 37°C, the suspension was removed and the monolayer was overlaid with 15 ml of EARLE's solution supplemented with 2 % of calf serum, 1 % of agar and 0.001 % of neutral red. The paper disk impregnated with a test material was placed on the solidified surface. After incubation for 48 hours at 37°C, the diameter of the cytotoxic zone and plaque inhibition zone were measured.



assay. The antibiotic was active agaist both herpes simplex virus strain HF and NDV in this assay system. Plaque-free protected zones were formed around the paper disc at low concentrations but inner cytotoxic zones appeared together with plaque-inhibited zones at higher concentrations. It was observed that a linear relationship existed between the logarithm of the concentration and diameter of plaque-inhibited or cytotoxic zones. The chemotherapeutic index is 8~16 in this assay system.

The cytotoxicity to HeLa cell culture was determined by a check for cytotoxicity through direct microscopic examination of treated cell layers after incubation periods of 1 to 7 days. The highest well-tolerated concentration by this observation was 0.25 mcg/ml; the cells became round and detached from glass surface at concentrations higher than this level. Therefore, a serial dilution experiments with treatments of 0.25 mcg/ml or less were carried out to determine the inhibitory dose. As demonstrated in Table 1, cytopathic effect (CPE) caused by infection of NDV was completely inhibited in triplicated tubes at a concentration of 0.25 mcg/ml. At half this concentration, however, CPE appeared to the same extent as in the control, although CPE was retarded for 2 days.

Table 2 shows the antiviral spectrum of trichothecin using the same cell host system as in Table 1. The antibiotic was effective against all RNA viruses tested but inactive against adeno virus type 5. The reasons why it was inactive are not clear. From the results in Tables 1 and 2, the chemotherapeutic index was approximately 1 in the tube culture assay system.

Table 2. Antiviral spectrum of trichothecin

Viruses	Inhibition of CPE	Effective concentration (mcg/ml)
Polio[1]	Positive	0.25
Measles[2]	Positive	0.25
NDV[3]	Positive	0.25
Adeno[4]	Negative	—
Vaccinia[5]	Positive	0.25
Herpes simplex[6]	Positive	0.25

Methods were the same as in Table 1.
1) Poliovirus type 1 strain CH.
2) Measles virus strain Sugiyama.
3) Newcastle disease virus strain Miyadera.
4) Adenovirus type 5 strain GO.
5) Vaccinia virus strain DIE.
6) Herpes simplex virus strain HF.

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Kei Arima
Akira Takatsuki
Seikichi Suzuki*
Kunio Ando
Gakuzo Tamura

Department of Agricultural Chemistry, The University of Tokyo
*Research Laboratory of Chugai Pharmaceutical Co., Ltd.

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