SCREENING OF MICROBIAL INHIBITORS OF MAMMALIAN ORNITHINE DECARBOXYLASE

Sir:

In the course of searching for inhibitors of mammalian ornithine decarboxylase (ODC), an active compound was isolated from the culture broth of an unidentified fungus and identified as citrinin, a known antibiotic[1,2]. Citrinin showed cytotoxicity *in vitro* to mouse leukemia cells. Structurally related compounds were examined for possible inhibition of ODC and cytotoxicity, and we found that N^{ω}-hydroxy-L-ornithine, a degradation product of vanoxonin[3], is an inhibitor of ODC.

ODC was prepared as follows at below 4°C unless otherwise mentioned. To a 8-week old female rat (SPF-Donryu) weighing 180 to 200 g, thioacetamide was administered at 150 mg/kg intravenously 24 hours before sacrifice. The liver was removed and homogenized in 0.25 M sucrose solution. The homogenate was centrifuged at $10,000 \times g$ for 20 minutes, and the supernatant was centrifuged again at $100,000 \times g$ for 60 minutes. The supernatant was used as crude enzyme for the screening. For kinetic analysis, the crude enzyme was purified 10-fold by successive application of DEAE-cellulose column chromatography, ammonium sulfate fractionation and dialysis. Protein was determined by the method of LOWRY *et al.*[4].

The reaction mixture contained the followings in 1 ml: 50 mM sodium phosphate (pH 7.2), 0.2 mM pyridoxal-5-phosphate, 5 mM dithiothreitol, 0.2 mM L-ornithine, D,L-[5-^{14}C]ornithine (0.05 μCi, Amersham International, plc), either inhibitor solution or water (100 μl) and enzyme solution (200 μl, added last). The reaction progressed for 90 minutes at 37°C, and was terminated by heating in a boiling water bath. After cooling, the reaction mixture was diluted with 4 ml of water and centrifuged at 2,000 rpm for 15 minutes. The supernatant was applied to a 0.5-ml column of Amberlite CG-50 (Na^+ type). Unreacted D,L-[5-^{14}C]ornithine was eluted first with 5 ml of 0.4 N NH_4OH, and thereafter [1-^{14}C]putrescine, the reaction product, was eluted with 2 ml of 5 N NH_4OH. The eluate of [1-^{14}C]-putrescine was collected in a vial, mixed with 6 ml of scintillation solution (Atomlight, New England Nuclear) and submitted to radioactivity measurement with a Beckman scintillation counter (Model LS9800). By this assay method, *Km* for ornithine was 0.4 mM.

Table 1. Inhibition of ODC by citrinin and N^{ω}-hydroxy-L-ornithine.

Compound	*Ki* (mM)
Citrinin	0.15
N^{ω}-Hydroxy-L-ornithine	0.04

Table 2. *In vitro* inhibition of the growth of mouse leukemia cells by citrinin and N^{ω}-hydroxy-L-ornithine.

Compound	IC_{50} (μg/ml)		
	L1210	L5178Y	P388
Citrinin	8.4	4.3	3.3
N^{ω}-Hydroxy-L-ornithine	44.0	*	*

* Not tested.

The fungus was grown in flasks with shaking at 180 rpm for 4 days at 27°C. The culture medium was composed of tomato paste 2.4%, dextrin 2.4%, yeast extract 1.2%, and $CoCl_2$ 0.0006% in distilled water (pH 7.0). The ODC inhibitory activity in the broth filtrate was precipitated at pH 2 and crystallized from methanol to obtain citrinin as yellow needles (250 mg per liter of the broth)[2].

Both citrinin and N^{ω}-hydroxy-L-ornithine inhibited rat liver ODC competitively with respect to ornithine, with *Ki* values of 0.15 mM and 0.04 mM, respectively (Table 1). Citrinin inhibited the growth of mouse leukemia cells L1210, L5178Y and P388 with IC_{50} values of 8.4, 4.3 and 3.3 μg/ml, respectively (Table 2). N^{ω}-Hydroxy-L-ornithine also inhibited the growth of L1210 cells with a IC_{50} value of 44.0 μg/ml (Table 2).

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