AN INHIBITOR OF CHLORAMPHENICOL ACETYLTRANSFERASE PRODUCED BY *STREPTOMYCES*

Sir:

It is known that bacterial resistance to chloramphenicol (CP) in clinical isolates is usually associated with the inactivation of CP by chloramphenicol acetyl-transferase (CAT). TANAKA and others[1] have reported on inhibition of CAT of *Escherichia coli* by basic triphenylmethane dyes. We obtained a substance, N-9174, exhibiting the inhibitory activity against the enzyme, from the culture broth of *Streptomyces* strain N-9174 which was isolated from a soil sample collected in Niigata Prefecture.

The activity of the inhibitor, N-9174, was determined biologically. CAT was prepared from *Streptococcus faecalis* N-117 as described in the previous paper[2].

A solution of 0.5 ml of 50 mM Tris-HCl buffer (pH 7.8) containing the inhibitor and 0.1 ml of CAT (0.17 units/mg) in the same buffer was placed in a test tube and was shaken at 37°C for 1 hour. To the mixture, 0.1 ml of CP (0.62 μmol/ml), 0.1 ml of acetyl CoA (1.24 μmol/ml) in the same buffer, and 0.2 ml of buffer were added. It was shaken continously for 1 hour and then heated at 100°C for 2 minutes in boiling water to stop the reaction. Controls without the inhibitor or CAT were prepared and were treated in the same way as the sample solution.

CAT inhibitory activity was estimated by measuring residual CP contents of the reaction mixture with the agar diffusion method using *Bacillus subtilis* ATCC 6633 as the test organism.

Streptomyces strain N-9174 producing the inhibitor was inoculated to a medium consisting of 2% glucose, 0.5% beef extract, 0.5% peptone, 0.2% $NaNO_3$, 0.1% K_2HPO_4, 0.05% KCl, 0.05% $MgSO_4 \cdot 7H_2O$ and 0.001% $FeSO_4 \cdot 7H_2O$, and shake-cultured at 30°C for 3 days.

The cultured broth was centrifuged at 8,000 rpm for 20 minutes. The supernatant was brought to 70% saturation of ammonium sulfate and the precipitate was collected and dialyzed overnight against 0.02 M Tris-HCl buffer (pH 7.8). The resulting extract was applied to DEAE-cellulose column and eluted with 0.01 M Tris-HCl buffer containing a linear gradient of zero to 1.0 M sodium chloride. The fractions with the highest activity were collected and applied to gel filtration on Sephadex G-100. The resultant pooled eluate with the activity was freeze-dried and stored at -10°C.

Purified N-9174 was colorless and its chemical reactions gave the following results: FEHLING, negative; SELIWANOFF, negative; BIAL, negative; ninhydrin, positive and FOLIN-CIOCALTEU, positive. Based on these data, N-9174 was considered to be a protein.

The activity of N-9174 was optimal at pH 7.8 and 40°C, and disappeared at 80°C for 10 minutes. However, the inhibitor was stable at 0~5°C for 2 years. Moreover, it was confirmed that this substance had no protease activity by the casein-agar method and no effect on acetyl CoA itself.

Purified N-9174 inhibited CAT (0.17 units/mg) from *Streptococcus faecalis* at a concentration of 200 μg/ml, and showed no antibacterial and no antifungal activity at 1 mg/ml. The intraperitoneal injection of 1 g/kg caused no toxic reactions in mice.

The effect of N-9174 on CP sensitivity of CP-resistant bacterial strains was tested. *Streptococcus faecalis* N-117, *Streptococcus haemolyticus* 0-78, *Streptococcus pneumoniae* N-77, *Proteus rettgeri* GN-624, *Klebsiella pneumoniae* GN-69, *Salmonella typhi* H-901 and *Escherichia coli* CP-65 producing CAT respectively were used as test organisms. The results, as shown in Table 1, indicated that the inhibitor enhanced the activity

Table 1. *In vitro* effect of N-9174 on the minimal inhibitory concentrations of chloramphenicol against chloramphenicol-resistant bacteria.

Organism	MIC (μg/ml)	
	Chloramphenicol	Chloramphenicol + N-9174 (1 mg/ml)
Streptococcus faecalis N-117	125	31.2
Streptococcus haemolyticus O-78	62.5	31.2
Streptococcus pneumoniae N-77	31.2	15.6
Proteus rettgeri GN-624	500	250
Klebsiella pneumoniae GN-69	2,000	250
Salmonella typhi H-901	250	62.5
Escherichia coli CP-65	2,000	125

of CP against all test strains, without reference to species and strains.

The *in vivo* effect of N-9174 on CP therapy of experimental streptococcal infection in mice was examined. Mice were infected intraperitoneally with 0.5 ml of an overnight broth culture of CP-resistant *S. haemolyticus* O-78, and were injected subcutaneously 6 hours later with 0.1 ml of CP (500 μg/ml), or 0.1 ml of CP and 0.1 ml of N-9174 (100 μg/ml) in separate sites. The treatments were continued once a day to each group consisting of 20 mice. As a result, it was observed that untreated mice died within 1 to 3 days and that CP-and-N-9174 therapy resulted in improved survival compared with CP therapy alone, as shown in Table 2.

Table 2. Effect of treatment with chloramphenicol and chloramphenicol+N-9174 on the survival time of mice inoculated with *Streptococcus haemolyticus* O-78

Group	Treatment[1]		Results	
	Dose		Average survival[2] (±SD)	ILS[3]
	ml/mouse	mg/kg		
Control			2.2±0.76	
Chloramphenicol	0.1	2.5	5.0±1.84	2.27
Chloramphenicol +N-9174	0.1+0.1	2.5+500	12.9±5.19	5.86

1) Treatment subcutaneously
2) Average survival day of 20 mice per group
3) Increase in life span over untreated controls

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