STUDIES ON THE ANTITUMOR ACTIVITY OF AN ALAZOPEPTIN ISOLATED FROM A NEW STRAIN OF *STREPTOMYCES*

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Recently, a streptomycete strain, No. OS-3256, was isolated from a soil sample collected in the suburbs of Tokyo. A culture filtrate of this strain indicated a marked survival effect (more than 200 %) on mouse leukemia L-1210. The effective component was extracted from the culture filtrate. It showed remarkable antitumor activity against mouse leukemia L-1210 and S-180 in mice. The physico-chemical properties of this compound are similar to those of the diazoketone compounds, and it was shown to be alazopeptin.[1]

Strain: The aerial mycelium of strain No. OS-3256 was branching, the sporophore was straight, representative of the section Rectiflexibiles of Pridham *et al.*[2] and the spore was oval or ellipsoidal with a smooth surface. The aerial mycelium belonged to the white color series of Tresner and Backus.[3]

Among known species of *Streptomyces*, *Streptomyces candidus* (Krasilnikov, 1941) Waksman, 1953[4] and *Streptomyces orientalis* Pittenger and Brigham, 1956[4] were considered to be related to strain No. OS-3256. However, *Streptomyces orientalis* was different from the strain in cultural characteristics on several identification media. Although the strain differed from *Streptomyces candidus* in peptonization of milk, utilization of carbohydrates and its products, it resembled *Streptomyces candidus* in cultural and morphological characteristics. Therefore, this strain, No. OS-3256, was considered to be a new variety of *Streptomyces candidus* and the name *Streptomyces candidus* var. *azaticus* nov. var. Awaya and Hata was proposed with reference to its products. The strain was also different from streptomycetes which produced azaamino acid antibiotics, as shown in Table 1.

Assay: The culture filtrate had scarcely any activity against gram-positive or gram-negative bacteria and weak antifungal activity against *Saccharomyces cerevisiae*, *Aspergillus niger* and *Screlotinia cinerea* on a natural medium. However, by the use of the chemically defined media of Davis and Mingioli[5], it is possible to detect a strong inhibitory activity against *Bacillus subtilis* PCI 219.

The antibacterial activity against *B. subtilis* PCI 219 on Davis' agar media paralleled the anti-L-1210 activity in mice.

Fermentation: The production of the effective substance determined by antibacterial and antitumor activities was examined. When the strain was incubated in a culture medium containing 2 % starch, 2 % soybean

Table 1. Comparison of *Streptomyces candidus* var. *azaticus* with azaamino acid antibiotic-producing *Streptomyces*

Strain	*Streptomyces candidus* var. *azaticus*	*Streptomyces griseoplanus*[5]	*Streptomyces fragilis*[6]	*Streptomyces ambofaciens*[7]
Antibiotic	Alazopeptin	Alazopeptin	Azaserine	DON Duazomycin A, B, C
Spore chain	Straight	Spirales	Spirales to straight	Spirales
Spore surface	Smooth	Warty	Smooth	Warty
Aerial mycelium	White	Gray	Yellow-pink	Gray
Reverse side of colony	Dull yellowish orange	Grayed yellow to yellow brown	Light grayish reddish brown	Colorless to grayed yellow
Melanoid pigment	Negative	Negative	Negative	Negative

meal and 0.3 % NaCl at 27~30°C, anti-L-1210 activity appeared in about 20 hours, reaching a peak in 30~50 hours.

Extraction: The culture broth was harvested after 30 hours of fermentation and filtered. The active component was adsorbed on a activated carbon, washed with water, and eluted with 20 % aqueous acetone. After concentration under reduced pressure, methanol was added, and the impurities which settled were eliminated. Freeze-drying produced a light yellow powder. The crude powder was purified by carbon and silica gel chromatography.

Physico-chemical properties: The product was colorless and amphoteric. It was unstable at pH 2.0, stable at pH 7.0, and slightly unstable at pH 9.0. The ultraviolet absorption spectrum showed maxima at about 244 nm and 275 nm. The diazo group in the molecule was confirmed by the strong absorption band at 2110 cm^{-1} in the infrared spectrum. The Rf value of this compound by paper chromatography of an ascending method, using a solvent system composed of 93.8 % *n*-butanol - 44 % propionate (1 : 1) was 0.45~0.5. In addition, the compound gave DON by an enzyme extracted from the mycelium of the strain OS-3256 and produced alanine by acid hydrolysis. Therefore, the product was identified as alazopeptin.

Antitumor activity:

(1) Effects on L-1210: CDF_1 mice, around 20 g in weight, with 6 mice per group, were used, and 10^5 cells of L-1210 were transplanted intraperitoneally. Starting after 24 hours, diluted solutions of the sample in various concentrations were injected intraperitoneally once a day until the animals were dead. As shown in Table 2, the effect of the sample was 287 % at 57.3 mg/kg/day with a remarkable survival rate. A better effect was obtained by daily administration than by intermittent administration every 2 days.

(2) Effects on S-180: A small slice (size 2 cubic mm) of S-180 solid tumor was implanted subcutaneously to the axillar region of *dd* mice. The sample was administered intravenously once a day beginning after 24 hours for 10 days. On the 11 th day, the tumor was taken out and its weight compared with that of the control. As shown in Table 3, T/C % showed that at 18.2 mg/kg/day, there was complete remission of the tumor, and at 9.1 mg/kg/day, 90 % remission.

Table 2. Effect of OS-3256 substance on L-1210*

Dose (mg/kg/day)	Route and regimen	Survivors	Body weight difference (g)	MST T/C (days)	Average response (%)
57.3**	i.p. Day 1~death	6/6	−4.0	23.0/8.0	287
28.7	〃	〃	−1.5	16.0/8.0	200
14.3	〃	〃	−2.0	14.0/8.0	175
7.2	〃	〃	+0.3	13.0/8.0	163
3.6	〃	〃	−1.5	11.0/8.0	138
1.8	〃	〃	0	8.5/8.0	107

* Inoculum size: 10^5 cells intraperitoneally.
** LD_{50} of the sample is more than 3 g/kg in mouse (i.p.)

Table 3. Effect of OS-3256 substance on S-180

Dose (mg/kg/day)	Route and regimen	Survivors	Body weight difference T/C (g)	Tumor weight T/C (mg)	Inhibition (%)
36.4***	Days 1~10*	10/10	−6.4/−1.0	0/1401	100
18.2	〃	〃	−3.3/−1.0	8/1401	99
9.1	〃	〃	−1.4/−1.0	158/1401	89
4.5	〃	〃	−2.2/−1.0	1102/1401	21
36.4	Days 5~11**	10/10	−5.1/−2.7	73/2500	97
18.2	〃	〃	−0.9/−2.7	259/2500	90
9.1	〃	〃	+2.4/−2.7	568/2500	77
4.5	〃	〃	−1.1/−2.7	1870/2500	25

* Mice were sacrified 11 th day after implantation.
** Mice were sacrified 12 th day after implantation.
*** LD_{50} of the sample is more than 1.5 g/kg in mouse (i.v.).

When treatment was started 5 days after the transplantation, there was still a marked antitumor activity.

(3) Effects on EHRLICH ascites carcinoma: A total of 10^7 cells of EHRLICH ascites carcinoma were injected to *dd* mice intraperitoneally. After 24 hours the sample was injected intraperitoneally, and the increase of ascites (increase in body weight) as well as the days of survival were determined. Moreover, during the treatment, ascites cells were removed and morphological changes in the tumor cells were checked by the Giemsa-stain method. No life prolongation effect or cytotoxic effect of the sample was observed with the carcinoma.

Summary

By screening for the antitumor agent produced by soil microorganisms, it was found that a culture filtrate of a new species of streptomyces named *Streptomyces candidus* var. *azaticus* nov. var. AWAYA and HATA had marked survival effcet in mouse leukemia L-1210. The effective compound was identified as alazopeptin.

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