LACTOQUINOMYCIN, A NOVEL ANTICANCER ANTIBIOTIC

I. TAXONOMY, ISOLATION AND BIOLOGICAL ACTIVITY

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Lactoquinomycin, a novel basic antibiotic, was isolated from the culture broth of a soil streptomyces by repeated solvent extraction and adsorption column chromatography. Morphological, cultural and physiological studies revealed that the organism belongs to the species *Streptomyces tanashiensis*. The antibiotic was active against bacteria, particularly Gram-positive organisms, and neoplastic cells *in vitro*. Antibiotic-resistant cell sublines of L5178Y lymphoblastoma were more significantly inhibited by lactoquinomycin than the parental cell line. Lactoquinomycin was effective against Ehrlich carcinoma in mice.

In the course of our screening program for new antitumor antibiotics, using drug-resistant neoplastic cells, we found that a soil actinomycete strain IM8442T produces a novel antibiotic, which inhibits antibiotic-resistant cell sublines of L5178Y murine lymphoma more markedly than the parental cells. The new agent was designated lactoquinomycin, following the chemical structure[1]. Taxonomy of the producing organism, isolation and some biological activities are presented in this publication.

Taxonomy of the Producing Organism

The organism was isolated from a soil sample, collected at Hiratsuka City, Kanagawa-ken, Japan, in 1984. Taxonomic studies principally followed the methods adopted by the International Streptomyces Project (ISP)[2].

Morphological Properties

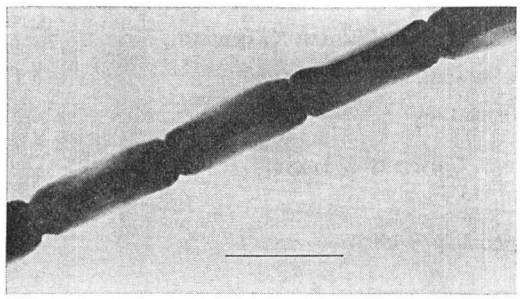
Microscopic and electron microscopic studies showed that fairly long, straight and slightly flexuous aerial mycelia were formed from the branched mycelia grown in yeast - malt agar and other agar media. Mature spore-chains were straight and slightly flexuous, and consisted of more than 20 spores per chain. The spore shape was cylindrical ($0.4\sim0.6\times1.0\sim1.3$ μm), and the spore surface was smooth, as seen by electron microscope (Plate 1). Typical whorls of barbed wire appearance and other special morphologies were not observed, although umbel-like structures of $3\sim6$ short branches were found in the poorly-growing culture on sucrose - nitrate agar and some other media.

Cultural Characteristics

For experiments on cultural properties, all the cultures were incubated at 27°C and observed on 3, 7, 14 and 21 days. The color recorded for the mature cultures was described according to the "Color Harmony Manual"[3]. The color of mature sporulated aerial mycelium was in the Gray series[4], but immature aerial hyphae displayed a yellowish or reddish tint. The reverse side of the colony had yellowish brown pigment which was a pH indicator changing to cherry pink in an alkaline solution. Melanoid pigment and yellowish brown diffusible pigment were produced in some media. The cul-

Plate 1. Electron microphotograph of spore chains of strain IM8442T (tyrosine agar, 27°C, 10-day culture).

The inserted scale is 1 μm.



tural characteristics on various media at 27°C for 2 weeks are summarized in Table 1.

Physiological Studies

The organism grew in a range of 10~37°C (temperature optimum 27~30°C) on yeast - malt agar, and in 20~37°C (optimum 20~30°C) on oatmeal agar.

The utilization of carbon compounds was examined by the method of PRIDHAM and GOTTLIEB[5]. The following carbohydrates supported growth as a sole carbon source in PRIDHAM-GOTTLIEB basal agar medium; D-glucose, L-arabinose, D-xylose, galactose and salicin (Table 2). Other physiological characteristics are summarized in Table 3.

The cell wall composition was analyzed by the method of BECKER *et al.*[6], showing LL-diaminopimelic acid. It was classified as Type I, indicating that strain IM8442T belongs to the genus *Streptomyces*.

Table 1. Cultural characteristics of strain IM8442T.

Medium	Growth	Aerial mycelium	Reverse	Soluble pigment
Sucrose - nitrate agar	Moderate	Poor, powdery; pale yellow (2ca)	Pale yellow (2ca)	None
Glucose - asparagine agar	Poor	Moderate, powdery; pale yellow (2ca)	White (a)	None
Glycerol - asparagine agar (ISP-5)	Moderate	Good, velvety; light brownish gray (3fe) & gray (d)	Dull yellow (2ne)	None
Inorganic salts - starch agar (ISP-4)	Good	Good, velvety; pale yellowish brown (2fe) & light brownish gray (3fe)	Pale yellowish brown (2ec)	None
Tyrosine agar (ISP-7)	Good	Abundant, floccus; light brownish gray (3fe) & pale yellowish brown (2fe)	Pale yellowish brown (2ie)	Dark brownish gray (3nl)
Nutrient agar	Poor	Poor, powdery; pale yellow (2db)	Pale yellow (1½ ca)	None
Yeast extract - malt extract agar (ISP-2)	Good	Good, velvety; light brownish gray (3fe), gray (d) & pale orange (5dc)	Grayish yellow brown (3ni)	Dark brownish gray (3nl)
Oatmeal agar (ISP-3)	Good	Good, powdery; light brownish gray (5fe)	Light brownish gray (3ge)	Dark brownish gray (2nl)

Incubation at 27°C for 2 weeks.
Color and number in parenthesis followed the color standard[3].

Table 2. Utilization of carbon sources by strain IM8442T.

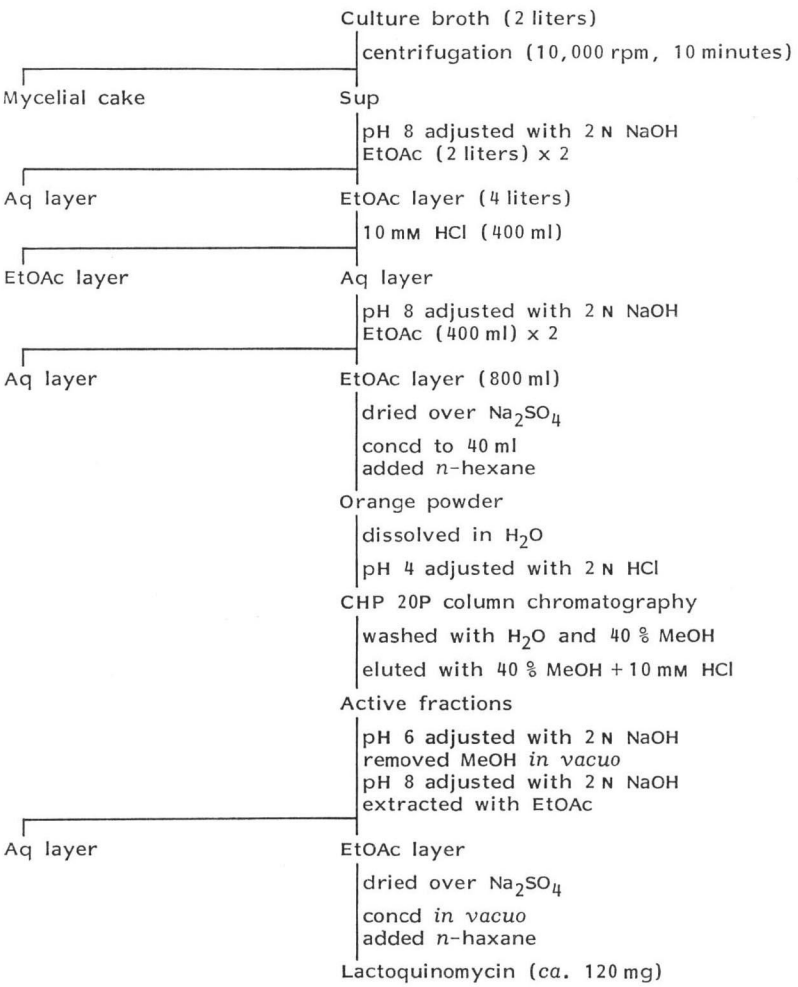
Growth	Carbon source
Positive	D-Glucose, L-arabinose, D-xylose, galactose, salicin
Negative	Sucrose, D-fructose, raffinose, rhamnose, inositol, D-mannitol

The basal medium used was ISP medium 9.

Table 3. Physiological properties of strain IM8442T.

Property		Medium
Starch hydrolysis	Positive	Inorganic salts - starch agar
Gelatin liquefaction	Positive	Glucose - peptone - gelatin
Milk coagulation	Negative	Skim milk
Milk peptonization	Positive	Skim milk
Melanoid pigment production	Positive	Tryptone - yeast extract broth, peptone - yeast extract - iron agar
H_2S production	Negative	

Fig. 1. Isolation scheme for lactoquinomycin.



The morphological, cultural and physiological characteristics of the organism, described above, revealed that strain IM8442T is related to *S. tanashiensis*, *S. nashvillensis* and *S. violaceorectus* according to BERGY's Manual of Determinative Bacteriology and KÜSTER's key for classification[7]. The comparative studies with ISP strains, ISP5195 (IMC S-0302), ISP5314 (IMC S-0342) and ISP5279 (IMC S-0349), revealed that strain IM8442T belongs to the species *S. tanashiensis*.

Table 4. Antimicrobial activity of lactoquinomycin.

Organism	MIC (μg/ml)	Organism	MIC (μg/ml)
Staphylococcus aureus FDA 209	1.56	*S. enteritidis* 11	100
Micrococcus luteus IAM 1056	6.25	*Shigella sonnei*	25
Bacillus subtilis PCI 219	3.13	*Proteus vulgaris*	100
B. cereus T	12.5	*Aspergillus niger* JIS 1-1	>100
Corynebacterium xerosis	3.13	*A. oryzae* IAM 2630	>100
Mycobacterium smegmatis R-15	>100	*Botrytis cinerea* IAM 5126	>100
M. phlei IAM 12064	50	*Mortierella ramannicinus* IAM 6128	>100
Escherichia coli B	100	*Penicillium chrysogenum* IAM 7326	>100
E. coli K 12	100	*Candida albicans*	>100
Pseudomonas aeruginosa IFO 3455	>100	*C. utilis* Y 21-6	>100
P. fluorescens H 3	>100	*Cryptococcus neoformans* IAM 122533	>100
Salmonella typhi	50	*Saccharomyces cerevisiae* Y23-9	>100

Bacteria: Mueller-Hinton agar medium (Difco) at 37°C.
Fungi: Yeast extract 0.2% - sucrose 1% agar medium at 27°C.

Fig. 2. Effect of lactoquinomycin on Ehrlich ascites carcinoma in ICR mice.

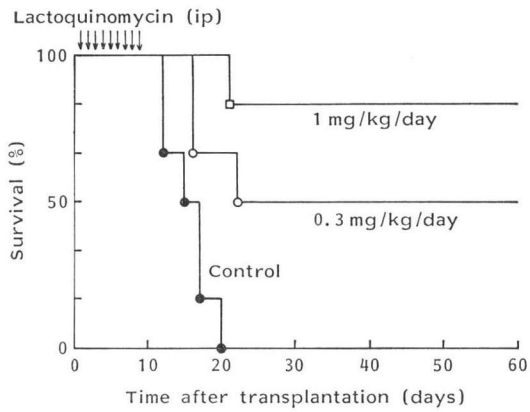


Table 5. Cytotoxicity of lactoquinomycin.

Cell line	IC_{50}* (ng/ml)
K562 human leukemia	33
L1210 murine leukemia	13
P388 murine leukemia	30
L5178Y murine lymphoma	
Parental	20
Adriamycin-resistant	6
Aclarubicin-resistant	13
Bleomycin-resistant	8

* The viable cell number was determined by the trypan blue dye exclusion method.

The media used were RPMI 1640 with 10% fetal calf serum for L1210 and K562 cells, RPMI 1640 with 10% fetal calf serum and 5 μM 2-hydroxyethyldisulfide for P388 cells, and RPMI 1640 with 10% horse serum for L5178Y cells. The cells of $1\sim2\times10^4$/ml were incubated with lactoquinomycin at 37°C for 72 hours in an atmosphere of 5% CO_2 and 95% air.

Production and Isolation of Lactoquinomycin

The vegetative inoculum was obtained by transferring spores of strain IM8442T to a 500-ml Sakaguchi flask containing 100 ml of a medium of oatmeal 2% and yeast extract 0.1%, pH 7.2. The inoculated medium was incubated at 27°C for 40 hours on a reciprocal shaker (150 rpm). For production of lactoquinomycin, *ca.* 2 ml of the inoculum growth was added to 100 ml of the same medium and incubated at 27°C for *ca.* 88 hours on a reciprocal shaker operating at 150 rpm. Antibiotic concentrations in fermentation and extraction samples were assayed by a disc method, using nutrient agar plates and *Bacillus subtilis* PCI 219 as a test organism.

The antibiotic in the culture fluid (2 liters) was extracted with ethyl acetate (2 liters×2) at pH 8.0, transferred to water layer (10 mM HCl, 400 ml), and again extracted with ethyl acetate (400 ml×2). Remaining water was removed by Na_2SO_4 from the ethyl acetate layer, which was then concentrated to 40 ml. An orange powder was obtained by addition of *n*-hexane to the concentrated solution. The powder was dissolved in water, and pH was adjusted to 4.0 with 2 N HCl. The antibiotic in the

solution was adsorbed on CHP 20P column, washed with water and then with 40% methanol, and eluted with 40% methanol containing 10 mM HCl. The active fractions were collected, pH was adjusted to 6.0, methanol was removed by evaporation *in vacuo*, pH was adjusted to 8.0, and the antibiotic was extracted with ethyl acetate. Lactoquinomycin, *ca.* 120 mg was obtained by addition of *n*-hexane by the procedure described above.

Antimicrobial Activity of Lactoquinomycin

Lactoquinomycin displayed inhibitory activity against bacteria, particularly Gram-positive organisms, but no significant activity against fungi. The minimal inhibitory concentrations (MIC) are presented in Table 4.

Antitumor Activity and Toxicity of Lactoquinomycin

Lactoquinomycin showed cytotoxicity for cell lines of K562 human myeloid leukemia, L1210 and P388 murine leukemia, and L5178Y murine lymphoblastoma in culture (Table 5). The 50% inhibitory concentrations, observed by the trypan blue dye exclusion method were in a range of 13~33 ng/ml. The antibiotic was more effective against adriamycin-[8], aclarubicin-[9] or bleomycin-resistant[10] cell subline of L5178Y lymphoma than the parental cell line.

When 10^6 cells of Ehrlich carcinoma were implanted ip into ICR mice and lactoquinomycin was injected ip everyday for 9 days starting 24 hours after the tumor transplantation, 1 mg/kg/day and 0.3 mg/kg/day inhibited the ascites increase and prolonged the survival period (Fig. 2).

The 50% lethal dose (LD_{50}) of lactoquinomycin for male *dd*Y mice was *ca.* 10 mg/kg by either intravenous or intraperitoneal injection.

Discussion

Lactoquinomycin is a novel antibiotic and the structure is assigned as shown in the subsequent paper[1]. The aglycon moiety shows the same structure with kalafungin. In spite of structural similarity, kalafungin displays antifungal activity but lactoquinomycin lacks in antifungal activity. Lactoquinomycin is related to medermycin but can be differentiated from medermycin by the physicochemical properties and antitumor activity[1,11]. Lactoquinomycin is rather stable but medermycin is labile. The former exhibits a marked anticancer activity, but the latter shows a very weak activity. The MICs are different[11]. Since detailed studies on medermycin structure have not been published, the comparison of the two substances is difficult. However, medermycin could be considered as an isomer of lactoquinomycin.

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