Communications to the Editor

ANGELMICINS, NEW INHIBITORS OF ONCOGENIC *src* SIGNAL TRANSDUCTION

Sir:

In the course of screening for new microbial substances which inhibit oncogenic signal transduction, we have found novel substances called angelmicins A and B from a culture of a rare actinomycete *Microbispora*, that were isolated from a soil sample collected at Mt. Tennyo, in Gunma Prefecture, Japan. In this communication, we report some of the physico-chemical properties and the biological activities of these new inhibitors.

Cell growth characteristics of transformed cells differ in many respects from those of normal counterparts. Transformed cells have a reduced serum or growth factor requirement for growth, and are able to grow in a defined mitogen-free medium because of their autocrine growth factor loops[1]. For growth of normal cells, on the other hand, a supplement of serum or growth factors is essential in culture. Using this difference in growth phenotypes between transformed and normal cells as a parameter, we recently established a method to identify specific inhibitors of oncogenic transformation[2]. In this assay system, such inhibitors should more strongly inhibit tumor cell growth in a serum-free medium than in a serum-containing medium. In the course of screening for such activity, a culture extract from a rare actinomycete *Microbispora* sp. AA9966 selectively inhibited the growth of *src* transformed cells in a serum-free medium. The active components A and B were purified using ethyl acetate extraction and Diaion HP-20 and YMC GEL ODS column chromatography. Angelmicins A and B had molecular weights of about 1,700 and their molecular formulae were determined as $C_{85}H_{112}O_{38}$ and $C_{85}H_{112}O_{37}$, respectively, based on FAB-MS and NMR spectra. The physico-chemical properties are described in

Table 1. Physico-chemical properties of angelmicins A and B.

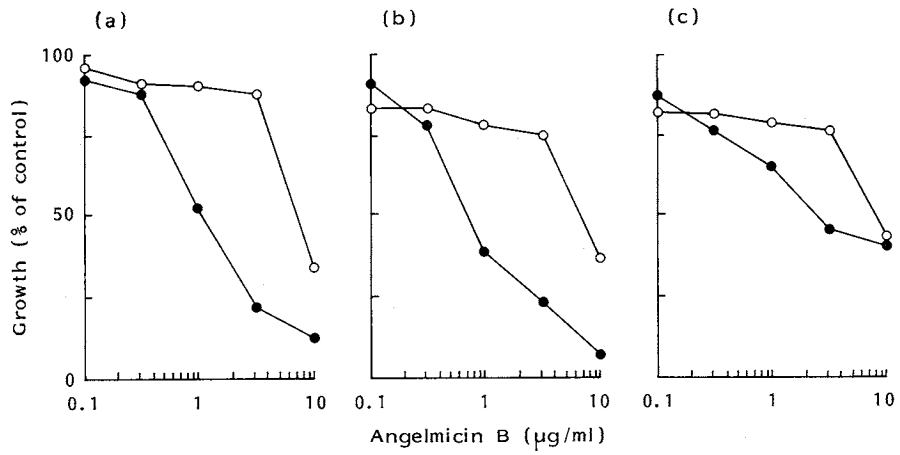
	Angelmicin A	Angelmicin B
Nature	Red powder	Red powder
MP (°C)	>200 (dec)	>200 (dec)
UV λ_{max} nm (ε)		
in MeOH	244 (38,700), 278 (46,400), 433 (15,900), 509 (10,000)	240 (41,200), 278 (43,800), 432 (13,900), 511 (10,800)
in 0.1 N HCl-MeOH (1:9)	239 (48,200), 281 (50,600), 433 (13,500), 510 (11,300)	239 (44,800), 281 (46,800), 431 (12,200), 510 (11,500)
in 0.1 N NaOH-MeOH(1:9)	236 (37,800), 278 (50,800), 437 (20,700), 613 (8,800), 648 (8,800)	235 (36,500), 278 (46,900), 436 (19,100), 614 (8,800), 647 (8,800)
IR ν_{max} (KBr) cm^{-1}	3450, 2940, 1700, 1620, 1455, 1405, 1120, 1055	3450, 2940, 1705, 1620, 1460, 1405, 1125, 1060
FAB-MS *m/z*		
Positive	1,764 $(M+H+Na)^+$	1,749 $(M+2H+Na)^+$
Negative	1,741 $(M+2H-H)^-$	1,725 $(M+2H-H)^-$
Molecular formula	$C_{85}H_{112}O_{38}$	$C_{85}H_{112}O_{37}$
Elemental analysis		
Calcd for	$C_{85}H_{112}O_{38}\cdot\frac{1}{2}H_2O$:	$C_{85}H_{112}O_{37}\cdot\frac{3}{2}H_2O$:
	C 58.31%	C 58.25%
	H 6.51%	H 6.61%
Found:	C 58.20%	C 58.45%
	H 6.70%	H 6.48%
	N <0.30%	N <0.30%
HPLC[a] Rt (minutes)	10.4	18.0
TLC[b] Rf	0.49	0.39

[a] (System 1) Column: Cosmosil 5C18 AR (4.6 mm i.d. × 150 mm, Nacalai Tesque, Inc.). Mobile phase: CH_3CN-H_2O (43:57). Flow rate: 1.2 ml/minute. Detection: UV absorption at 254 nm.

[b] (System 2) Plate: TLC plate RP-18 F_{254S} (Merck, Art 15423). Solvent: MeOH-H_2O (87.5:12.5).

Fig. 1. Selective growth inhibition of tyrosine kinase oncogene-transformed cells by angelmicin B in serum-free medium.

○ +Serum, ● −serum, (a) NIH3T3/*src*, (b) NIH3T3/*abl*, (c) NIH3T3/*ras*.



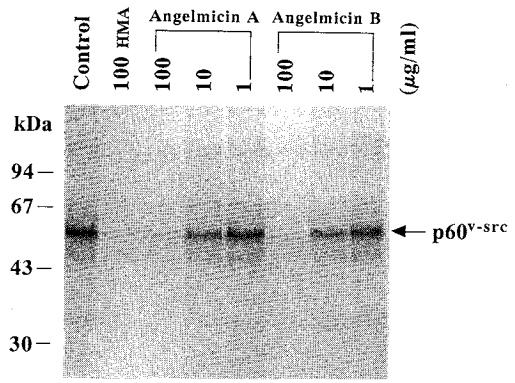
src, *abl* or *ras* oncogene transformed NIH3T3 cells were seeded in triplicate in 96 well plates precoated with fibronectin at a density of 5×10^3/well with serum-free or serum-containing medium as described elsewhere[2]. Angelmicin B was dissolved in DMSO and added to media at a final DMSO concentration of less than 0.1%. DMSO without any test compound was added to control wells. Cells were cultured for 3 days and quantitated by crystal violet staining. Growth rates are presented as percentages of those in control wells.

Table 1. They were soluble in dimethyl sulfoxide, methanol, acetonitrile, acetone and dichloromethane, but insoluble in *n*-hexane or water. Structural analyses suggested that the antibiotics contained one anthraquinone and six sugar moieties in each molecule.

The inhibitory activity on oncogenic signal transduction was examined using mouse NIH3T3 cell lines transformed by various oncogenes[3]. Angelmicin B specifically inhibited the growth of *abl* as well as of *src* transformed cells at a concentration range from 0.5 to 5.0 μg/ml in a defined serum-free medium, whereas its inhibitory effect on the growth of *ras* transformed cells in this medium was not significant (Fig. 1). The IC_{50} values shifted 7-, 10- and 2.5-fold between serum-free and serum-containing media corresponding to *src*, *abl* and *ras* transformed cells, respectively. Doxorubicin, vinblastine and other cytotoxic antitumor drugs showed no shift in IC_{50} values[2]. Thus, it appeared that the substance was selectively active against tyrosine kinase oncogene-transformed cells in reverting the transformed phenotype of low serum requirement to the normal cell phenotype.

To further evaluate the activity of angelmicins, we examined the *in vitro* effect on $p60^{v\text{-}src}$ autophosphorylation. $p60^{v\text{-}src}$ immunoprecipitates were prepared from lysates of *src* transformed NIH3T3 cells. As shown in Fig. 2, angelmicins A and B reduced the autophosphorylating activity of the $p60^{v\text{-}src}$ tyrosine kinase in a dose dependent manner. In another assay system in which activities

Fig. 2. Effect of angelmicins on $p60^{v\text{-}src}$ tyrosine kinase.



Immune complexes prepared from *src* transformed NIH3T3 cells were treated with indicated concentrations of angelmicins, and then assayed for $p60^{v\text{-}src}$ autophosphorylation activity as previously described[5]. The phosphorylation was analyzed by SDS-PAGE (9% gel) and autoradiography. Herbimycin A (HMA) was used as a positive control in the assay. Positions of $p60^{v\text{-}src}$ and molecular markers are as shown.

of various protein kinases can be determined simultaneously[4], it was demonstrated that angelmicin B selectively inhibited tyrosine kinase activity without affecting protein kinase C or protein kinase A (data not shown). We therefore conclude that angelmicins are specific tyrosine kinase inhibitors.

Acknowledgment

This research was supported in part by a Grant-in-Aid for Special Project Research on Cancer Bio-Science from the Ministry of Education, Science and Culture of Japan.

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(Received March 23, 1993)

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