COMMUNICATIONS TO THE EDITOR

Adxanthromycin: A New Inhibitor of ICAM-1/LFA-1 Mediated Cell Adhesion from *Streptomyces* sp. NA-148

Sir:

Intercellular adhesion molecule-1 (ICAM-1: CD54) and lymphocyte function-associated antigen-1 (LFA-1: CD11a/CD18) are cell surface adhesion molecules that interact with one another to promote a number of cellular interactions, including antigen-specific T lymphocyte stimulation[1,2] and leukocyte adhesion to endothelium followed by emigration into sites of inflammation[3,4]. In a number of *in vivo* studies, treatment of animals with monoclonal antibodies directed against either ICAM-1 or LFA-1 resulted in inhibition of the inflammatory process[5~9]. These results suggest that an inhibitor of ICAM-1 or LFA-1 could be a potential therapeutic drug for inflammatory and immunological diseases. Although the inhibition of ICAM-1/LFA-1 mediated adhesion of JY and HeLa cells by plant extracts has been reported[10,11], there is no report on inhibitors from microbial metabolites. A search for inhibitors of the ICAM-1/LFA-1 system from microbial metabolites was carried out using two methodologies: a homotypic cell aggregation assay and a cell adhesion assay. During the screening program, we found a new inhibitor, designated adxanthromycin, in the cultured broth of strain *Streptomyces* sp. NA-148. In this communication we describe the screening, isolation, structure, and biological properties of adxanthromycin.

JY Epstein-Barr virus-transformed B lymphoblastoid cells were used for the homotypic cell aggregation assay[12]. The assay was performed as the first screen for cell adhesion inhibitors. JY cells suspended in RPMI 1640 medium (Nikken Bio Medical Lab., Kyoto, Japan) were settled in flat-bottomed 96-well microtiter plates. The cells were stimulated with 50 ng/ml of phorbol-12-myristate-13-acetate (PMA) with or without test samples, then incubated at 37°C for 24 hours. Cell aggregation was observed microscopically, and cytotoxicity of the samples was also determined simultaneously by a calorimetric analysis using WST-1[13]. The culture without inhibitors showed large and compact aggregates, and the culture showed smaller clusters and non-aggregated cells in the presence of inhibitors. TS1/22 (anti-LFA-1 monoclonal antibody, ATCC, Manassasm VA) inhibited the formation of clusters or aggregates completely at 1 μg/ml. The samples which inhibited the formation of cell aggregates without cytotoxicity were further tested in the adhesion assay using soluble ICAM-1 and LFA-1 positive SKW-3 T cell leukemia cells[14]. SKW-3 cells (Hayashibara Biochemical Laboratories Inc., Fujisaki Cell Center, Okayama, Japan) were labeled with a fluorescence dye, BCECF-AM [2′,7′-bis-(2-carboxylethyl)-5-(and-6)-carboxylfluorescein, acetoxymethyl ester][15] and then stimulated with 20 ng/ml of PMA at 37°C for 20 minutes. The cell suspension was added to a 96-well microtiter plate coated with immobilized soluble ICAM-1 and incubated in the presence of inhibitors at 37°C for 30 minutes. The adherent cells were solubilized with 0.1% Nonidet P-40 and fluorescence intensity was measured.

The producing strain identified as *Streptomyces* sp. NA-148 was found by screening a total of 2450 cultures of Actinomycetes. The strain was cultivated in a 100-ml test tube containing 10 ml of TN-2 medium (glycerol 2.0%, soybean powder 1.5 %, $CaCO_3$ 0.3%, KH_2PO_4 0.01%, $Na_2HPO_4 \cdot 12H_2O$ 0.04%, $MgSO_4 \cdot 7H_2O$ 0.05%, pH 7.0) at 27°C for 3 days on a rotary shaker (230 rpm). The seed culture was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of the same medium, then cultured at 27°C for 5 days on a rotary shaker (180 rpm). The whole culture was transferred into a 5-liter mini-jar fermentor containing 3 liters of the same medium and cultured at 27°C for 9 days (agitation; 250 rpm, aeration; 3 liters/minute). The fermentation broth (24 liters) was filtrated and the mycelial cake was extracted with acetone. After removal of the acetone, the aqueous solution was extracted with EtOAc. The extracts was subjected to silica gel chromatography with $CHCl_3$-MeOH as the developing solvent to obtain a crude powder. The powder was purified by preparative HPLC (Senshu Pak ODS-H-5251) with MeOH-10 mM phosphate buffer, then washed with an acidic aqueous solution, isolating adxanthromycin (29 mg) as a pale yellow powder. The molecular formula, $C_{42}H_{40}O_{17}$ [$(M-H)^-$, m/z 815.2252] was established by high-resolution FAB-MS measurement, which was supported by ^1H and ^{13}C NMR spectral data. The physico-chemical properties of adxanthromycin were as follows, mp. 233~235°C (dec.), $[\alpha]_D$ +120.5° (c 0.20, DMSO), UV λ_{max} in MeOH (ε)

272 nm (5,770), 315 nm (6,730) and 358 nm (4,390) and λ_{max} in 0.1 N NaOH-MeOH (ε) 254 nm (9,310), 315 nm (6,400), 377 nm (8,170). The structure of adxanthromycin was deduced from NMR experiments including DQF-COSY, NOE difference, HMQC and HMBC. Adxanthromycin possesses a dimeric anthrone peroxide structure[16] glycosidated with α-galactose, as shown in Fig. 1. The ^{13}C NMR spectrum of adxanthromycin is shown in Fig. 2. The ^{1}H and ^{13}C NMR assignments (in DMSO-d_6 + HCl) for adxanthromycin are indicated belows: C-1 (δ_C 138.7), C-2 (δ_C 129.1), C-3 (δ_C 155.8), C-4 (δ_C 110.7/δ_H 7.27), C-4a (δ_C 148.9), C-5 (δ_C 117.0/δ_H 6.29), C-6 (δ_C 136.3/δ_H 7.07), C-7 (δ_C 114.4), C-8 (δ_C 159.6), C-8a (δ_C 124.9), C-9 (δ_C 189.6), C-9a (δ_C 122.0), C-10 (δ_C 80.0), C-10a (δ_C 141.6), C-11 (δ_C 20.4/δ_H 2.69), C-12 (δ_C 168.2), C-13 (δ_C 15.3/δ_H 2.19), C-14 (δ_C 33.2/δ_H 1.39), C-8-OH (δ_H 13.36), C-1′ (δ_C 139.5), C-2′ (δ_C 127.5), C-3′ (δ_C 158.1), C-4′ (δ_C 112.3/δ_H 7.20), C-4a′ (δ_C 148.9), C-5′ (δ_C 117.0/δ_H 6.28), C-6′ (δ_C 136.1/δ_H 7.06), C-7′ (δ_C 114.6), C-8′ (δ_C 159.6), C-8a′ (δ_C 125.1), C-9′ (δ_C 189.5), C-9a′ (δ_C 120.3), C-10′ (δ_C 79.7), C-10a′ (δ_C 141.2), C-11′ (δ_C 20.7/δ_H 2.68), C-12′ (δ_C 168.9), C-13′ (δ_C 15.3/δ_H 2.19), C-14′ (δ_C 32.9/δ_H 1.40), C-8′-OH (δ_H 13.48), C-1″ (δ_C 96.8/δ_H 5.73), C-2″ (δ_C 67.9/δ_H 3.95), C-3″ (δ_C 69.3/δ_H 3.74), C-4″ (δ_C 68.2/δ_H 3.75), C-5″ (δ_C 72.5/δ_H 3.55), C-6″ (δ_C 59.8/δ_H 3.24, 3.45).

Adxanthromycin inhibited the formation of the JY cell agrregates from 1.5 μg/ml in a dose dependent manner. The toxicity (IC_{50}) of adxanthromycin against JY cell was 15.2 μg/ml. Adxanthromycin inhibited SKW-3 adhesion to soluble ICAM-1 in a dose-dependent manner with an IC_{50} of 18.8 μg/ml. The cell toxicity (IC_{50}) of adxanthromycin against SKW-3 was 110.0 μg/ml. The inhibitory activity of the compound in the cell-free receptor binding assay was examined in the manner reported previously[17]. Adxanthromycin showed weak inhibition in the assay with an IC_{50} of 760 μg/ml in the cell-free system. Details of the screening method, biological activity and structure determination of adxanthromycin will be reported later.

Fig. 1. Structure of adxanthromycin.

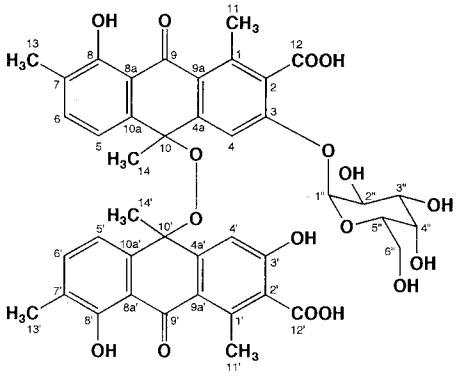
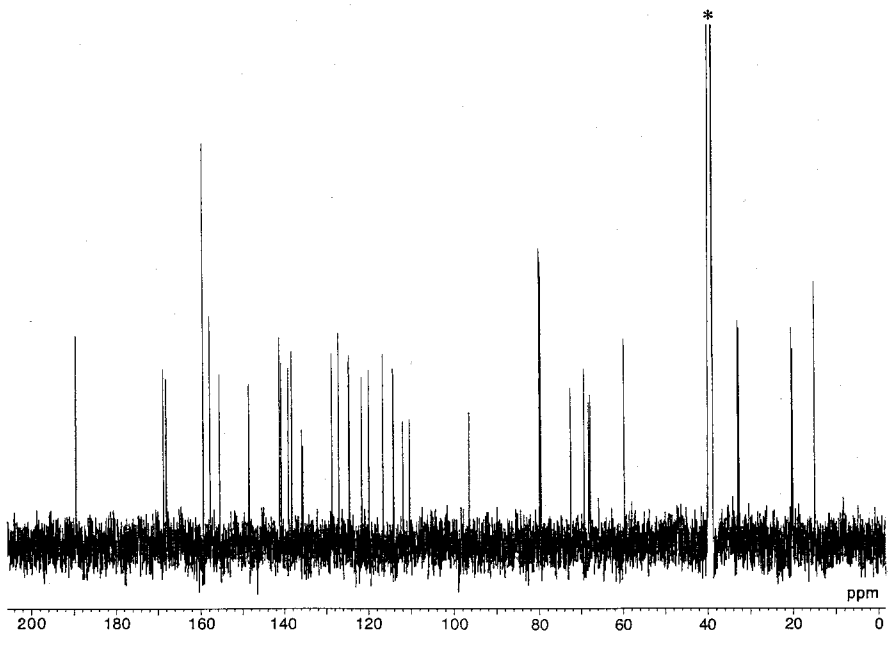


Fig. 2. ^{13}C-NMR spectrum of adxanthromycin (125 MHz, in DMSO-d_6 + HCl).



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(Received October 8, 1998)

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