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COMMUNICATIONS TO THE EDITOR

Isolation and Biological Activity of a Novel Cytokine Modulator, Cytoxazone

Sir:

Upon stimulation with antigen, naive $CD4^+$ cells can differentiate into distinct subsets defined by their cytokine secretion pattern. Human allergen-specific Th cells generally belong to the Th2 phenotype and produce IL-4, IL-5, IL-6, IL-10, and IL-13.[1~3] In contrast, Th1 cells, which are generally implicated in cellular immune response, produce IL-2, GM-CSF, INF-γ, and TNF-β.[1~3] Expansion of allergen-specific Th2 cells has been shown to associate with pathophysiologic disorders observed in atopic patients, including patients with atopic dermatitis, whose skin is infiltrated by large numbers of allergen-specific Th2 cells.[4,5] Therefore, inhibitors of Th2-dependent cytokine production would be potent chemotherapeutic agents in the field of immunotherapy.

In the course of our screening for chemical immunomodulators from microbial metabolites, we found that an actinomycete strain (RK95-31) isolated from a soil sample collected in Fukuyama City, Hiroshima Prefecture, Japan, produced a novel immunosuppressant, cytoxazone (Fig. 1). The strain RK95-31 was identified to be *Streptomyces* sp. and deposited in the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan, with the accession No. FERM P-16171.

The cytoxazone producing organism was cultured in the 70 ml seed medium consisting of 2% glucose, 2.5% soybean meal, 1% soluble starch, 0.4% yeast extract, 0.1% meat extract, 0.2% NaCl, 0.005% K_2HPO_4, 0.05% $CaCO_3$, and 0.003% Popon-S™ (adjusted at pH 7.2 before sterilization). The seed culture was carried out on a rotary shaker at 250 rpm at 28°C for 48 hours. Then, 70 ml of the culture was inoculated in a 30-liter jar fermenter containing 18 liters of the same medium with 0.05% of CA-123 and KM-68 antifoam. The fermentation was carried out at 28°C for 96 hours under constant agitation at 300 rpm and aerated 7 liters per minute. The fermentation broth was centrifuged to remove mycelia, and the supernatant was extracted with EtOAc at pH 7.0. The EtOAc layer was concentrated *in vacuo*, the remaining oily material was chromatographed on a silica gel column with 0~50% methanol in chloroform. Cytoxazone was eluted with 5% methanol in chloroform, followed by purification on HPLC using a reverse phase column (PEGASIL ODS, 20 mm × 250 mm) eluting isocratically with 30% aqueous methanol. Finally the recrystallization was carried out in 30% aqueous methanol to give 140 mg of cytoxazone.

Cytoxazone is a colorless crystal with melting point of 118~121°C, optically active with $[\alpha]_D^{23}$ −71° (*c* 0.1, MeOH). The molecular formula was determined to be $C_{11}H_{13}NO_4$ by HR-MS (FAB, positive) (Found: *m/z* 224.0958 $(M+H)^+$. Calcd: *m/z* 224.0923). UV spectrum has absorption maxima (ε) at 225 (19600), 277 (2710), and 284 (sh, 2300) in MeOH. The IR spectrum (KBr) showed absorption bands at 3475, 3250, 2950, 1715, 1615, 1515, 1400, 1250, 1050, and 995 cm^{-1}. The ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) spectra in DMSO-d_6 of cytoxazone are shown in Figs. 2 and 3. The structure of cytoxazone was determined to be (4*R*,5*R*)-5-hydroxymethyl-4-(4-methoxyphenyl)-2-oxazolidinone by NMR spectral analyses including pulse-field gradient HMBC spectroscopies, X-ray crystallographic analysis, and CD spectra. Structural elucidation studies in detail will be reported in another paper. Cytoxazone has a novel structure containing a unique 2-oxazolidinone moiety, which is rare in microbial metabolites.

At first, we investigated the effect of cytoxazone on cytokine-production in whole spleen cells of normal BALB/c mouse. Cytoxazone at concentrations of 6.25 to 25 μg/ml significantly inhibited the IL-4- and IL-10-production induced by pokeweed mitogen (PWM)-treatment in whole spleen cells (Fig. 4). Since cytoxazone did not inhibit the PWM-induced GM-CSF production, we examined the effect of cytoxazone to cloned Th1 and Th2 type cells.[6] S38-9 (Th1 type) and S11-7 (Th2 type) cells were cloned from TCR$\alpha\beta$ positive T cells in spleen

Fig. 1. Structure of cytoxazone.

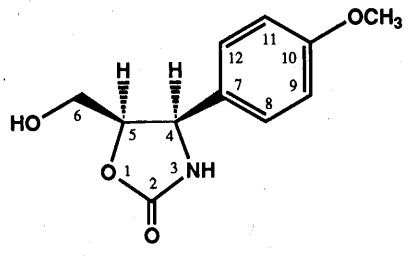


Fig. 2. ^{1}H-NMR spectrum of cytoxazone (600 MHz, in DMSO-d_6).

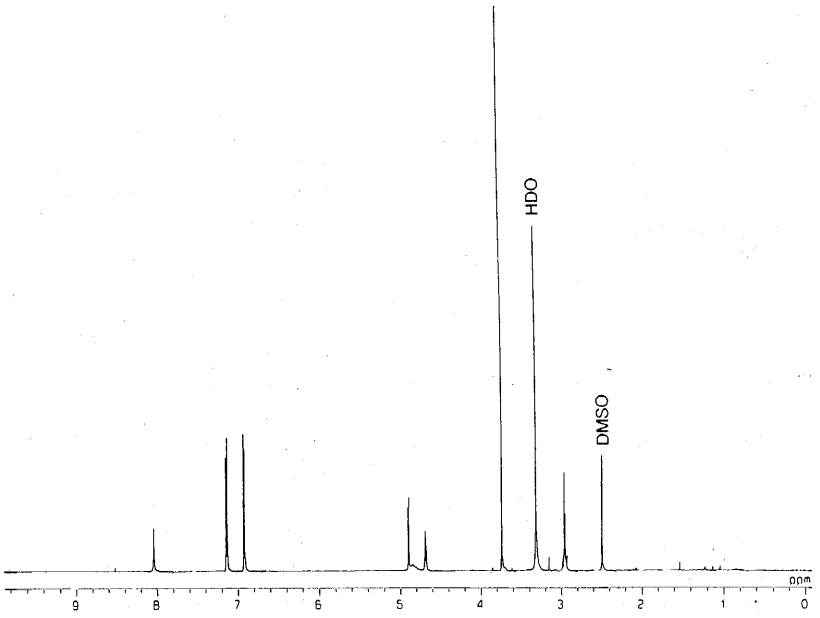
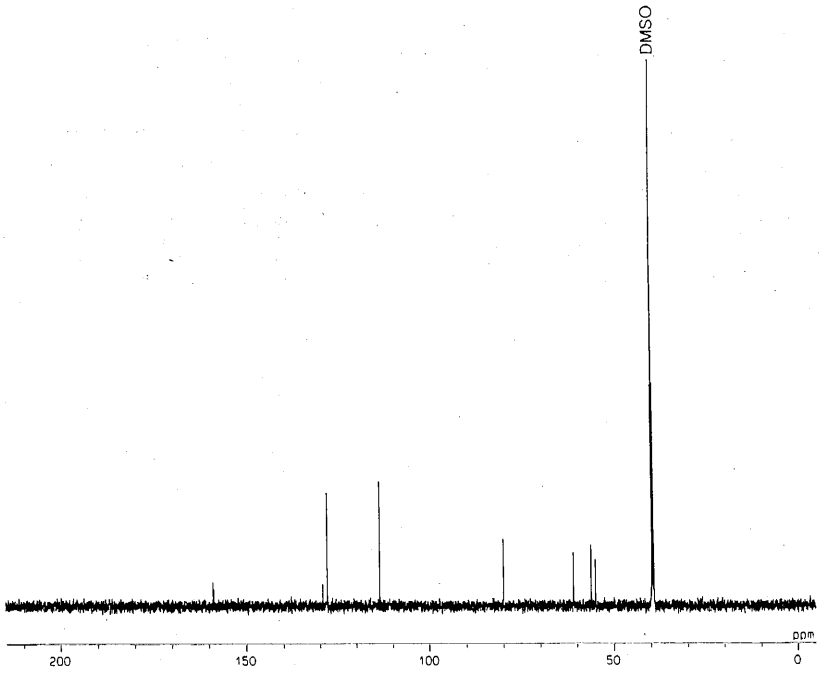
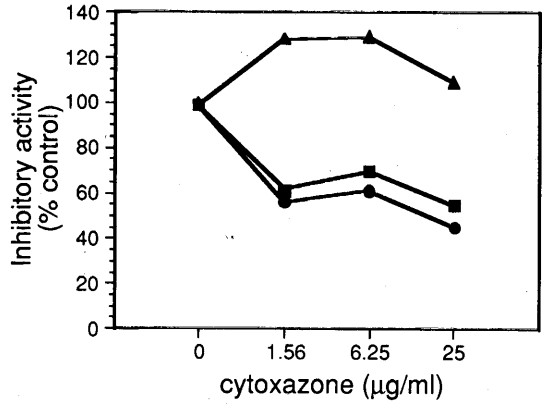


Fig. 3. ^{13}C-NMR spectrum of cytoxazone (150 MHz, in DMSO-d_6).



cells of CDF1 mice immunized with membrane antigen designated as CDL-1 prepared from fibrosarcoma cells. Th1 and Th2 types were determined according to a profile of cytokine production. Cytoxazone inhibited the IL-4- and IL-10-production from Th2 type clone, S11-7 cells stimulated by PWM at the same concentration range but did not inhibit the GM-CSF-production from Th1 type clone, S38-9. Growth inhibitory effect of cytoxazone on these Th1 and Th2 cells was not observed at a concentration of 100 μg/ml. These results suggest that cytoxazone inhibits the cytokine production *via* the signaling pathway of Th2 cells, but not Th1 cells. Further

Fig. 4. Inhibition of PWM-induced IL-4 and IL-10 productions in whole spleen cells of normal BALB/c mouse.



Spleen cells (2.5×10^6) taken from BALB/c mice were cultured at 37°C in 5% CO_2 humidified atmosphere in the presence of indicated concentrations of cytoxazone and 5 μg/ml PWM in RPMI1640 including 10% fetal bovine serum and 50 μM 2-mercaptoethanol. After the incubation for 48 hours, the released IL-4, IL-10, and GM-CSF in the conditioned medium were measured by the sandwich ELISA method. ▲, GM-CSF production; ■, IL-4 production; ●, IL-10 production.

studies on the biological mechanism of cytoxazone are in progress.

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