Novel Neuritogenic Activities of Pseurotin A and Penicillic Acid

Sir:

Neurotrophic factors (NTF) are known to be essential for the survival and functional maintenance of nerve cells in the central and the peripheral nervous system[1~3]. The decrease in availability of NTF is considered to cause dysfunction of the nervous system, resulting in various neural diseases including senile dementia such as ALZHEIMER's disease. Compounds which regulate the synthesis and secretion of neurotrophic factors might be useful as pharmaceutical agents for treatment of various neurodegenerative diseases involving dementia. In the search for potential compounds which modulate nerve growth factor (NGF) synthesis or mimic NGF (induce cell differentiation), staurosporine[4], lactacystin[5], BU-4514N[6] and epolactaene[7] have been isolated from microbial culture broth. During the course of our screening for microbial metabolites which induce cell differentiation of PC12 cells, rat pheochromocytoma cell line, pseurotin A[8] and penicillic acid[9] (Fig. 1) were isolated. Although the structures of these compounds are known, it is noteworthy report their biological properties because of their potential usefulness. We report here production, isolation and biological activities of these compounds.

PC12 pheochromocytoma cell has been shown to be a useful model cell for the study of adrenergic neuronal differentiation[10]. PC12 cells were cultured in DMEM medium containing 10% fetal bovine serum and 10% horse serum, in a humidified atmosphere of 5% CO_2 in air at 37°C. The cells were inoculated into 96-well collagen coated microtiter plate and pre-incubated for 1 day, then test samples were added to the cultures. After 2-days incubation, neurite-like structure inducing activities were monitored by microscopic observation. In this assay, β-NGF showed strong neurite-like structure inducing activity at concentrations above 50 ng/ml, and induced multipolar and branching type of neurite (Fig. 2B). Neurite formation in PC12 cells was also induced by 100 μg/ml of dibutyryl cAMP and by 25 μg/ml of forskolin, but these agents induced dipolar type of neurite. Staurosporine (0.02 μg/ml) had a strong neurite formation activity, but was toxic to the cell body. Therefore, we selected the samples which induced neurite-like structure similar to β-NGF and exhibited low toxicity.

After screening of microbial culture filtrates, we isolated pseurotin A and penicillic acid. For isolation of pseurotin A, *Diheterospora chlamydosporia* NF-1150 was cultured in media containing glucose 1%, sucrose 2%, soy bean meal 2%, KH_2PO_4 0.1% and $MgSO_4 \cdot 7H_2O$ 0.05% for 4 days at 25°C on a rotary shaker. The active principle was extracted from broth filtrate (7 liters) with EtOAc and successively purified by silica gel column chromatography ($CHCl_3$ - MeOH = 20 : 1) and Sephadex LH-20 column chromatography (MeOH). UV, FAB-MS, ^1H and ^{13}C NMR data revealed that it was identical to pseurotin A. It had already been recognized as a metabolite of *Pseudeurotium ovalis* with an unusual heterospirocyclic system[11]. For isolation of penicillic acid, *Penicillium* sp. FJ-256 was cultured in the same media for 4 days at 25°C on a rotary shaker. The active principle was successively purified by Diaion HP-20 column chromatography (50% aqueous MeOH) and Sephadex LH-20 column chromatography (MeOH). UV, FAB-MS, ^1H and ^{13}C NMR data revealed that it was identical to penicillic acid[12]. It had been isolated as an antibiotic substance produced by fungi.

Pseurotin A induced neurite-like structure in the

Fig. 1. Structures of pseurotin A and penicillic acid.

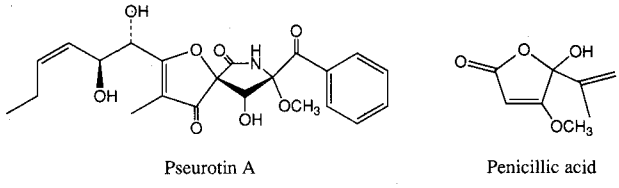
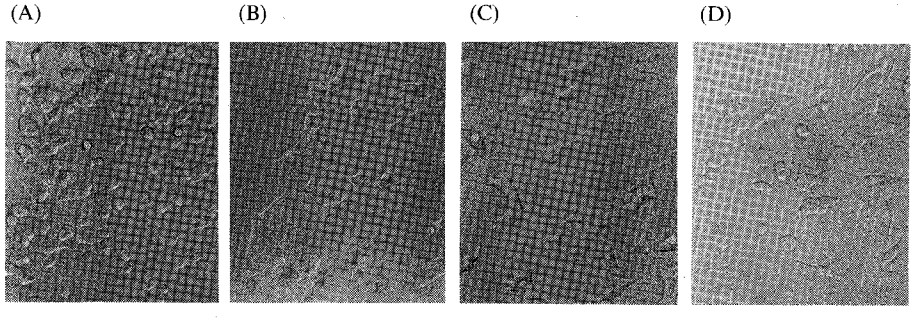


Fig. 2. Morphological changes of PC12 cells treated with pseurotin A, penicillic acid and β-NGF.



The cells were cultured at 37°C in 5% CO_2 humidified atmosphere in DMEM medium containing 10% fetal bovine serum and 10% horse serum on collagen coated microtiter plate.

Photograph were obtained after 2 days cultivation with or without agents. (A) control; (B) with 50 ng/ml of β-NGF; (C) with 20 μg/ml of pseurotin A; (D) with 5 μg/ml of penicillic acid.

morphology of PC12 cells and its neurite was multipolar type like β-NGF. The control PC12 cells cultured without drugs extended quite a few neurites. When the cells were treated with pseurotin A at concentrations ranging from 0.4 to 25 μg/ml, many neurites were extended from the cell bodies in a dose-dependent manner (Fig. 2C). Pseurotin A significantly increased neurite outgrowth both in number and length. At concentrations higher than 30 μg/ml, pseurotin A exerted a cytotoxic effect. The IC_{50} value of pseurotin A was 12 μg/ml in A2780 human ovarian carcinoma cell.

On the other hand, the IC_{50} value of penicillic acid was 1.5 μg/ml in A2780 human ovarian carcinoma cell. Penicillic acid induced neurite-like structure in the morphology of PC12 cells but its neurite was dipolar type like dibutyryl cAMP. When the cells were treated with penicillic acid at concentrations ranging from 3 to 6 μg/ml, many neurites were extended from the cell bodies (Fig. 2D). Penicillic acid was more toxic for PC12 cells than pseurotin A.

Therefore, we think pseurotin A may be a useful tool to investigate the mechanism of neurite formation of neuronal cells. It may also possess neuroprotective properties that are useful in the treatment of diseases involving the dysfunction of the nervous system due to deficiency of neurotrophic factor and was provide an important lead for treating neuroblastoma. Interestingly enough, lactacystin, BU-4514N, epolactaene and pseurotin A have a related partial structure. All these compounds possess a α-acyltetramic acid or β-hydroxy-γ-lactam moiety. We think penicillic acid also has a similar partial structure. The structure-activity relationships of these compounds may prove to be interesting in terms of mechanism of action and will be described elsewhere.

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References

1) PEREZ-POLO, J. R.: Neuronotrophic factors. *In* Cell Culture in Neurosciences. *Eds.*, J. E. BOTTENSTEIN & G. SATO, pp. 95~123, Plenum Press, 1985
2) HEFTI, F. & W. J. WEINER: Nerve growth factor and Alzheimer's disease. Ann. Neuro. 20: 275~281, 1986
3) BARDE, Y.-A.: Trophic factors and neuronal survival. Neuron 2: 1525~1534, 1989
4) MORIOKA, H.; M. ISHIHARA, H. SHIBAI & T. SUZUKI: Staurosporine-induced differentiation in a human neuroblastoma cell line, NB-1. Agric. Biol. Chem. 49: 1959~1963, 1985
5) ŌMURA, S.; T. FUJIMOTO, K. OTOGURO, K. MATSUZAKI, R. MORIGUCHI, H. TANAKA & Y. SASAKI: Lactacystin, a novel microbial metabolite, induces neuritogenesis of neuroblastoma cells. J.Antibiotics 44: 113~116, 1991
6) TODA, S.; S. YAMAMOTO, O. TENMYO, T. TSUNO, T. HASEGAWA, M. ROSSER, M. OKA, M. Y. SAWADA, M. KONISHI & T. OKI: A new neuritogenetic compound BU-4514N produced by *Microtetraspora* sp. J. Antibiotics 46: 875~883, 1993
7) KAKEYA, H.; I. TAKAHASHI, G. OKADA, K. ISONO & H. OSADA: Epolactaene, a novel neuritogenic compound in human neuroblastoma cells, produced by a marine fungus. J. Antibiotics 48: 733~735, 1995
8) BLOCH, P.; C. TAMM, P. BOLLINGER, T. J. PETCHER & H. P. WEBER: Pseurotin, a new metabolite of *Pseudeurotium ovalis* Stolk having an unusual hetero-spirocyclic system. Helv. 59: 133~140, 1976
9) BIRKINSHAW, J. H.; A. E. OXFORD & H. RAISTRICK: Penicillic acid, a metabolic product of *Penicillium puberulum* Baibier and *P. cyclopium* Westing. Biochem. J. 30: 394~411, 1936
10) GREENE, L. A. & A. S. TISCHLER: Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which responds to nerve growth factor. Proc. Natl. Acad. Sci. U.S.A. 73: 2424~2428, 1976
11) BLOCH, P. & C. TAMM: Isolation and structure of pseurotin A, a microbial metabolite of *Pseudeurotium ovalis* Stolk with an unusual heterospirocyclic system. Helv. 64: 304~315, 1981
12) AMSTUTZ, E. D.; E. A. FEHNEL & C. R. NEUMOYER: The synthesis of benzene derivatives structurally similar to penicillic acid. J. Am. Chem. Soc. 68: 349~354, 1946