Topical Anti-inflammatory Activity of Dianemycin Isolated from *Streptomyces* sp. MT 2705-4

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In order to develop new anti-inflammatory agents having different action mechanisms compared with nonsteroidal and steroidal anti-inflammatory drugs, the culture broths of various actinomycetes isolated from soil were screened using an *in vivo* mouse ear edma assay and one strain (*Streptomyces* sp. MT 2705-4: KCTC 8651P) was selected. Activity-guided purification led to the isolation of a polyether compound, dianemycin. Topically, dianemycin showed a potent anti-inflammatory activity in mouse ear edema induced by croton-oil or arachidonic acid. ED_{50} value of dianemycin was found to be 0.8 mg/ear compared to 0.4 mg/ear of prednisolone in croton-oil ear edema. However, dianemycin did not show the inhibitory activity in UV-erythema and delayed hypersensitivity reaction. These results indicate that dianemycin is a potential topical anti-inflammatory agent.

Keywords : Dianemycin, *Streptomyces*, Topical, Anti-inflammatory activity, Ear edema, UV-erythema, Delayed hypersensitivity

INTRODUCTION

Until now, nonsteroidal (NSAIDs) and steroidal anti-inflammatory drugs (SAIDs) are widely used in various inflammatory diseases. However, NSAIDs occasionally show the serious adverse effects such as stomach ulcer and kidney failure (Settipane, 1981), and long-term use of SAIDs produces Cushing's syndrome (Kim, 1990). In addition, several inflammatory/immunoregulatory disorders (psoriasis and *rheumatoid arthritis*) can not be effectively treated using these conventional drugs. Therefore, new anti-inflammatory agents are needed to overcome these problems. New drugs should have different action mechnisms compared to those of NSAIDs and SAIDs. To elucidate this possibility, we have screened the anti-inflammatory activity of the culture broths of actinomycetes isolated from soil using an *in vivo* mouse ear edema assay. In this study, dianemycin was successfully isolated as an active principle from one of the actinomycetes isolates, *Strepotomyces* sp. MRT 2705-4. Dianemycin was previously isolated as a polyether antibiotic by Hamill *et al.* (1969).

In this investigation, the topical anti-inflammatory activity of dianemycin was examined. Dianemycin was found to possess the potent topical anti-inflammatory activity in animal models of cutaneous inflammation.

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MATERIALS AND METHODS

Dianemycin was isolated from the culture broth of *Streptomyces* sp. MT 2705-4 (deposited in Korean Collection for Type Cultures, KRIBB, Taejon, Korea). *Streptomyces* sp. MT 2705-4 was cultured in the antibiotic fermentation medium (1.0% soluble starch, 2.0% glucose, 2.5% soybean meal, 0.1% meat extract, 0.4% yeast extract, 0.4% NaCl, 0.2% K_2HPO_4) at 28°C for 5 days. After centrifugation of culture broth, supernatant was extracted with ethylacetate. The ethylacetate fraction was evaporated and loaded onto silica gel column using chloroform:methanol (100:1) as a mobile phase. The fractions were combined and evaporated to dryness. The dried residue was further purified with Sephadex LH-20 column chromatgraphy using chloroform:methanol (1:1). The detailed isolation procedure, and chemical and physical data of dianemycin were described previously (Mheen *et al.*, 1990). The chemical structure of dianemycin was shown in Fig. 1.

Male ICR mice (20±2 g) and SD rats (200±20 g) were maintained in our animal facility (22±1°C) and

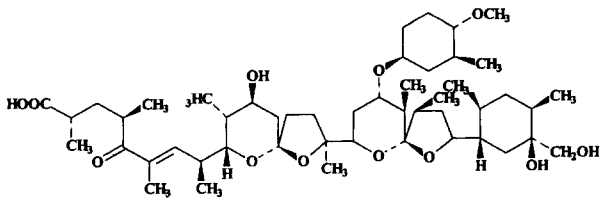

Fig. 1. Chemical structure of dianemycin.

acclimatized at least 3 days prior to use. Animals were fed with standard lab. pellet chaw (Sam Yang Co., Seoul, Korea) and water *ad libitum*. Croton-oil, arachidonic acid, prednisolone and indomethacin were purchased from Sigma Chem. Co. (St. Louis, MO USA). Picryl chloride was purchased from Nacalai tesque (Kyoto, Japan).

Dianemycin was tested in the following animal models of inflammation. Mouse ear edema assay was carried out by slight modification (Kim *et al.*, 1993) of an original procedure of Tonneli *et al.* (1965). The UV-induced erythema test was performed using rats by the procedure of Law and Lewis (1977). The delayed hypersensitivity reaction was induced by topical application of picryl chloride following the procedure of Tarayre *et al.* (1990). In all tests, dianemycin was topically applied to ears of mouse or abdomen of rats. Statistical significance was evaluated using Student's t-test and less than $P<0.05$ was regarded as a significant difference.

RESULTS AND DISCUSSION

In this investigation, dianemycin isolated from *Streptomyces* sp. MT 2705-4 was evaluated for the topical anti-inflammatory activity using animal models of cutaneous inflammation. We used four different animal models of inflammation in which dianemycin was treated topically to mice and rats. In croton-oil induced ear edema, dianemycin showed the potent anti-inflammatory activity as shown in Table I. Its ED_{50} value was 0.8 mg/ear compared to an ED_{50} value of prednisolone (0.4 mg/ear), one of the potent steroidal anti-inflammatory drugs. In the arachidonic acid induced ear edema assay, dianemycin was also found to be active. The anti-inflammatory activity was comparable to that of indomethacin. These results indicated that dianemycin might show the broad anti-inflammtory activity via topical route of administration, since it is known that inflammagens used, croton-oil and arachidonic acid, induced cutaneous inflammation via different mechanisms (protein kinase C activation in case of croton-oil and eicosanoid generation in case of arachidonic acid) as suggested by several authors (Tadimeti *et al.*, 1993; Young *et al.*, 1984; Lubach and Kietzmann, 1992). However, dianemycin did not show any anti-inflammatory activity against the UV-induced erythema test as well as the delayed type hypersensitivity test at the doses tested (Table II and III). When indomethacin was topically administrated at the indicated doses, indomethcin showed potent anti-inflammatory activity against the UV-induced erythema as reported by Buclier *et al.* (1990). Prednisolone also showed potent inhibition in delayed hypersensitivity at 0.25 mg/kg (44.7% inhibition). Delayed hypersensitivity reaction is known to be induced by the sensitized lymphocytes (T-cells) and many immunosuppressive drugs inhibit this reaction. The fact that dianemycin had no activity in this model might be well correlated with our finding that dianemycin did not inhibit T-cell proliferation at concentrations up to 0.1 ug/ml by the method described previously (Namgoong *et al.* 1994). Therefore, it is suggested that dianemycin possesses the different action mechanism compared with NSAID or SAID. Actually, dianemycin, one of polyether antibiotics, is reported to behave as a membrane perturbator (Hamill *et al.*, 1969). But, the detailed action mechanism remains to be elucidated. In our preliminary study, dianemycin did not show any acute toxicity in mice up to 10 mg/ear by topical application (data not shown). From all of these

Table I. Inhibition of mouse ear edema by dianemycin

Groups[a]	Dose (mg/ear)	Ear thickness[b] increased, mm		Ear thickness[c] increased, mm	
Control	-	0.17±0.03	(-)[d]	0.16±0.02	(-)
Prednisolone	0.1	0.11±0.02**[e]	(33)	NT[f]	
	0.3	0.09±0.02**	(45)	NT	
	0.9	0.06±0.02**	(66)	NT	
	2.0	0.04±0.01***	(74)	NT	
Indomethacin	0.1	NT		0.11±0.02	(33)
	0.9	NT		0.05±0.02***	(69)
Dianemycin	0.03	0.16±0.03	(6)	0.17±0.03	(-)
	0.1	0.13±0.02	(24)	0.18±0.04	(-)
	0.3	0.10±0.02**	(43)	0.16±0.02	(-)
	0.9	0.08±0.02***	(56)	0.10±0.02*	(37)
	2.0	0.07±0.01***	(59)	0.09±0.03*	(41)

Data were represented in mean±SD. [a]All compounds were topically applied to ears of mice (n=6), 30 min before the treatment of inflammagen. [b]Croton-oil incuced ear edema assay. [c]Arachidonic acid induced ear edema assay. [d]Values in parenthesis represent % inhibition. [e]*$P<0.05$, **$P<0.01$, ***$P<0.001$, significantly different from the corresponding control groups. [f]Not tested.

Table II. Inhibition of UV erythema by dianemycin

Groups	Dose (mg/rat)	Inhibition score
Control	-	0
Indomethacin	0.75	3
	1.5	4
	3.0	4
Dianemycin	1.0	0
	5.0	0

All compounds were topically applied to the shaved abdomen of rats (n=5), 30 min before the UVB-irradiation for 10 min.

Table III. Inhibition of delayed hypersensitivity by dianemycin

Groups	Dose (mg/ear)	Thickness increased, mm	% inhibition
Control	-	0.17±0.05	
Prednisolone	2×0.25	0.09±0.01*	44.7
Dianemycin	2×0.03	0.16±0.03	6.0
	2×0.06	0.18±0.06	-
	2×0.12	0.17±0.03	-
	2×0.25	0.16±0.02	6.0

All compounds were topically applied to ears of mice, 30 min before the treatment of picryl chloride and 30 min before the second treatment of picryl chloride to ears of mice (n=8). *: P <0.05, significantly different from the control group.

findings, dianemycin appears to be a potential topical anti-inflammatory agents having antimicrobial action.

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