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Macrolides with Promotive Activity of Monocyte to Macrophage Differentiation

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Abstract We have been interested in the immunomodulatory effect, to promote differentiation of the human monocytic cell line THP-1 to macrophages of EM-A. We chemically modified EM-A in order to obtain derivatives with stronger promoting activity of monocyte to macrophage differentiation and no antibacterial activity. Most of the EM701 derivatives produced, all 12-membred macrolides, were remarkably active and were free of antibacterial activity. Among them, the most potent derivative was EM703, which showed very weak gastrointestinal motor-stimulating activity. EM703 may be useful tool to study the mechanisms of action of macrophage differentiation, and may be lead candidate for the development of new therapeutic drugs for chronic airway disease.

Keywords macrolide, 12-membered macrolide, novel actions, immunomodulatory effect, anti-inflammatory effect, macrophage differentiation

Macrolide antibiotics are widely used as antimicrobial agents. Previously, we discovered strong gastrointestinal motor-stimulating (GMS) activity by erythromycin A (EM-A) and its non-antimicrobial derivatives [1, 2], and the generic name ‘motilide’ was proposed for a series of macrolides having motilin-agonistic activity [3～6].

Recently, EM-A and other macrolide antibiotics have been shown to be efficacious against chronic inflammatory airway disease, in addition to having antibacterial activity. The prognosis for diffuse panbronchiolitis (DPB), an incurable chronic inflammatory airway disease, improved significantly in two studies upon treatment with long term and low doses of such macrolide antibiotics [7, 8]. Such therapeutic efficacy is thought to be caused by either anti-inflammatory or immunomodulatory activity of macrolide antibiotics.

EM-A and azithromycin (AZM), one of the 15-membered ring macrolide antibiotics, have been shown to have inhibitory activity against the inflammatory functions of neutrophils, *in vitro* [9]. The two antibiotics also have a clinical effect in the treatment of DPB. Furthermore, EM-A has exhibited a prophylactic effect on lung injury *in vivo* in a bleomycin-induced acute lung injury rat model [10].

We previously clarified the suppressive effect of interleukin (IL)-8 release in a human bronchial epithelial cell line by EM-A, clarithromycin (CAM) and EM-derivatives [11]. We also examined the anti-inflammatory

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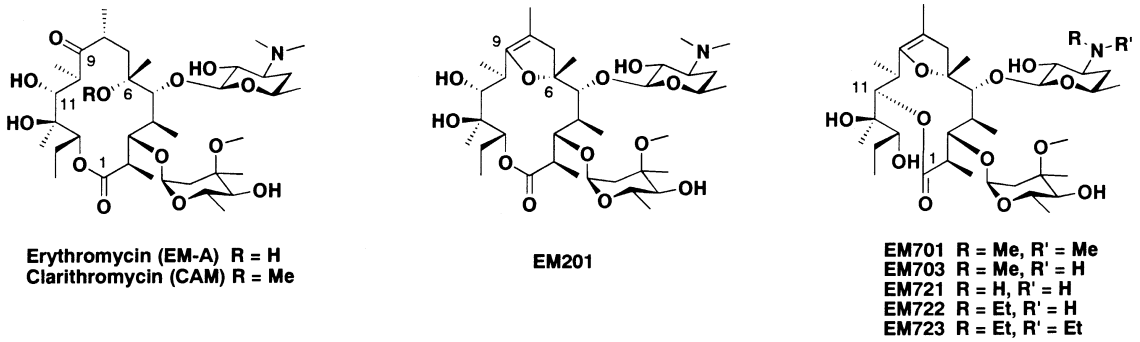

Erythromycin (EM-A) R = H
Clarithromycin (CAM) R = Me

EM201

EM701 R = Me, R' = Me
EM703 R = Me, R' = H
EM721 R = H, R' = H
EM722 R = Et, R' = H
EM723 R = Et, R' = Et

Fig. 1 Structures of erythromycin A, its derivatives and clarithromycin.

Table 1 Biological activities of EM-A, CAM and EM derivatives

Compounds	THP-1/Mϕ differentiation (promotive ability[a]; μM)					Antimicrobial activity[c] (MIC; μg/ml)				
	0.3	1	3	10	30	SA[d]	BS	BC	EC	KP
EM-A[e]	NT[b]	−	−	−	±	0.2	0.1	0.1	12.5	6.25
CAM[e]	NT	NT	−	−	+	0.1	0.1	0.1	6.25	6.25
EM201	NT	−	+	++	NT	50	25	25	>100	>100
EM701	NT	−	±	+	NT	>100	>100	>100	>100	>100
EM703	+	+	+	+	NT	>100	>100	>100	>100	>100
EM721	NT	NT	−	+	NT	>100	>100	>100	>100	>100
EM722	−	+	+	++	NT	>100	>100	>100	>100	>100
EM723	−	+	+	++	NT	>100	>100	>100	>100	>100

[a] Strength of activity was graded into four groups based on the ratio to 100 μM EM-A activity.
−: 0∼25%, ±25∼50%, +: 50∼100%, ++: >100%

[b] Not tested

[c] In this part of the data, MIC activities were obtained from our previous reports.

[d] SA: *Staphylococcus aureus* ATCC 6538P, BS: *Bacillus subtilis* ATCC 6633, BC: *Bacillus cereus* IFO 3001, EC: *Escherichia coli* NIHJ, KP: *Klebsiella pneumoniae* ATCC 10031

[e] EM-A: erythromycin A, CAM: clarithromycin

effects of EM-A, CAM, roxithromycin (RXM) and non-antimicrobial EM-derivatives by examining their inhibitory activity against rat leucocyte chemotaxis [12]. EM-A, CAM and RXM exhibited only weak chemotaxis inhibitory activity, while the non-antimicrobial EM-derivatives showed remarkable activity. Thus, the structural factors involved in antibacterial and anti-inflammatory activity are distinct.

On the other hand, we also reported on the immunomodulatory effect, to promote differentiation of the human monocytic cell line THP-1 to macrophages, of EM-A, CAM, RXM and EM-derivatives with no or weak antimicrobial activity *in vitro* [13, 14].

We believe that non-antibacterial EM derivatives could have various immunopharmacological activities, and that analysis of the mechanisms of action involved should be useful for development of new therapeutic drugs for chronic airway disease.

On the basis of the above findings, we chemically modified EM-A in order to obtain derivatives with stronger promoting activity of monocyte to macrophage differentiation and no antibacterial activity.

The promotive activities were determined by modifying the method of Keicho *et al.* (1994) [13]. THP-1 cell line, derived from a patient with monocytic leukemia, was supplied by the Japanese Cancer Research Resources Bank (Tokyo, Japan. Now the Japan Health Sciences Foundation).

THP-1 cells (1×10^5 per well in 0.5 ml) were seeded into 48-well tissue culture microplates (IWAKI, Japan) and cultured in the presence of phorbol myristate acetate (PMA; 2 ng/ml), each macrolide compound (0.3∼30 μM) alone, or

with macrolide compound and PMA, for 4 days at 37°C under 5% CO_2 humidified air. The number and viability of adherent cells were measured by colorimetric determination of MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl) tetrazolium bromide) at 550 nm.

Of the erythromycin derivatives, 8,9-anhydroerythromycin A 6,9-hemiketal (EM201), obtained by mild acid treatment of EM-A showed a promotive effect on THP-1 cell differentiation at 10 μM, and weak antimicrobial activity [14]. Next, EM201 was treated with K_2CO_3 in MeOH to afford the 12-membered macrolide, pseudoerythromycin A (EM701) [15]. Furthermore, de-*N*-methyl (EM703), and bis-de-*N*-methyl (EM721) derivatives were obtained by treating EM701 with I_2/NaOAc, and I_2/NaOMe [16]. *N*-Ethyl (EM722), and bis-*N*-ethyl (EM723) derivatives were obtained by treating EM721 with EtI/diisopropylethylamine.

Most of the EM701 derivatives, 12-membred macrolides listed in Table 1, were remarkably active and were free of antibacterial activity. Among them, the most potent derivative was EM703, which showed very weak gastrointestinal motor-stimulating activity.

Furthermore, the preliminary results indicated that EM703 exhibited a prophylactic effect on lung injury *in vivo* against a bleomycin-induced acute lung injury rat model, similar to EM-A.

In conclusion, the EM701 derivatives illustrated here may alone be useful tools to study the mechanisms of action of, and may be lead candidates for the development of, new therapeutic drugs for chronic airway disease.

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