VOL. 53 NO. 10, OCT. 2000 THE JOURNAL OF ANTIBIOTICS pp. 1219 — 1222

Effect of 14-Membered Ring Macrolide Compounds on Rat Leucocyte Chemotaxis and the Structure-activity Relationships

MASAKO OOHORI, KAZUHIKO OTOGURO, TOSHIAKI SUNAZUKA, SUZUKI KUNIHIKO, YUZURU IWAI and SATOSHI ŌMURA*

Research Center for Biological Function, The Kitasato Institute, and Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8642, Japan

(Received for publication June 5, 2000)

Diffuse pan bronchiolitis (DPB) is a common inflammatory airway disease in Asian countries[1,2]. Recently, the prognosis for DPB has been improved significantly by long-term and low-dose treatment of 14-membered ring macrolide antibiotics such as erythromycin (EM), clarithromycin (CAM) and roxithromycin (RXM)[3,4].

Anti-inflammatory mechanisms of macrolide antibiotics include the down-regulation of inflammatory cytokines and lymphocytes. EM and azithromycin (AZM), a 15-membered ring macrolide antibiotic that has clinical effect in the treatment of DPB, were shown to inhibit neutrophil functions in human peripheral blood of chemotaxis and active oxygen generation *in vitro*[5]. EM was also shown to exhibit a prophylactic effect on lung injury *in vivo* by using a bleomycin-induced acute lung injury model in rats[6].

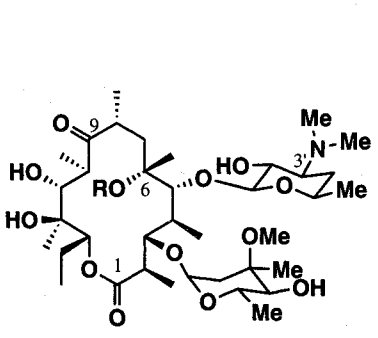
Previously, we discovered a strong gastrointestinal motor-stimulating (GMS) activity of EM and its non-antimicrobial derivatives[7,8] and proposed the generic name 'motilide' for a series of macrolides with this motilin-agonistic activity[9~12]. Furthermore, we studied the suppressive effect of a pre-inflammatory cytokine, interleukin (IL) -8 release of EM, CAM and eight EM-derivatives in a human bronchial epithelial cell line[13]. IL-8 release was potently suppressed by CAM and two EM derivatives that had no or weak antibacterial activity.

Here we studied the anti-inflammatory effects of EM, CAM, RXM, and four non-antimicrobial EM-derivatives (Fig. 1) by examining inhibitory activity on rat leucocyte chemotaxis and describe the structure-activity relationships.

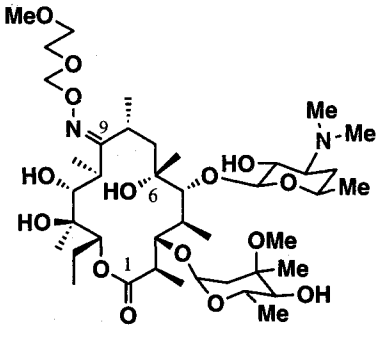
Male 6~8 week old Sprague-Dawley rats were injected with 10 ml of saline containing 1% of casein intraperitoneally. After 16~18 hours, the ascites and erythrocytes were removed by hypotonic lysis. Leucocytes, thus obtained, were suspended in RPMI-1640 medium with 10% FBS to make a suspension of 2×10^6 cells/ml for preincubation mixture.

Seven 14-membered ring macrolide compounds, EM, CAM, RXM, 8,9-anhydroerythromycin A 6,9-hemiacetal (EM201), de(*N*-methyl)-8,9-anhydroerythromycin A 6,9-hemiacetal (EM522), de(*N*-methyl)-*N*-isopropyl-8,9-anhydroerythromycin A 6,9-hemiacetal (EM574), and 8,9-

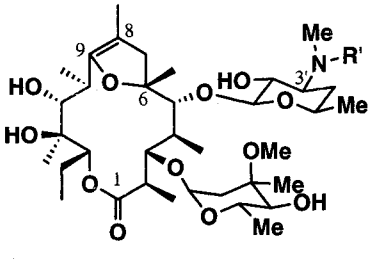
Fig. 1. Structures of erythromycin (EM), clarithromycin (CAM), roxithromycin (RXM) and EM-derivatives.



Erythromycin A (EMA) R = H
Clarithromycin (CAM) R = Me



Roxithromycin A (EMA)



EM201	**R'= Me**
EM522	**R'= H**
EM536	**R'= Me, -CH≡C. Br**
EM574	**R'= $CH(CH_3)_2$**

Dedicated to the memory of Sir EDWARD ABRAHAM.

anhydroerythromycin A 6,9-hemiacetal propargyl bromide (EM536) were dissolved in dimethyl sulfoxide (DMSO) to make solutions of 0.2, 2 and 6 mM. Each macrolide solution was added to make final concentrations of 1,10 and 30 μM. The final concentration of solvent DMSO was adjusted to 0.5% so that leucocytes would not be damaged. The suspension of leucocytes was preincubated for 60 minutes at 37°C under 5% CO_2 in humidified air before chemotaxis with macrolide compounds or DMSO for control and *N*-formyl Met-Leu-Phe (fMLP) at a final concentration of 10^{-8} M for positive control.

Leucocyte chemotaxis was assayed by a modified Boyden-chamber method using a 96-well chemotaxis chamber and polycarbonate filter with 3 μm pore size. The medium with fMLP at a final concentration of 2×10^{-8} M and macrolide compounds were poured into the wells of lower chamber. Two hundred μl of preincubated leucocytes suspension was poured into the wells of the upper chamber at a density of 4×10^5 cells.

The chamber was incubated for 2 hours at 37°C under 5% CO_2 in humidified air. Then, the supernatant was removed from the upper chamber and 200 μl of 2 mM EDTA solution in PBS (−) was poured into wells to stop migration.

After the chamber was incubated at 5°C for 20 minutes, the lower chamber was centrifuged and the number of leucocytes that migrated through the filter was measured by colorimetric determination of MTT assay at 550 nm.

The inhibition rate of leucocyte chemotaxis was calculated from following equation. "Sample abs." is the absorbance of the macrolide compound added to the well. "0% release abs." is the absorbance that was executed with no fMLP in the lower chamber, and "fMLP 10^{-8} M abs." is the absorbance of the fMLP added to the well at the final concentration of 10^{-8} M instead of the macrolide sample.

$$\text{inhibition rate (percent)} = 100 - \frac{(\text{Sample abs.} - 0\%\ \text{release abs.}) \times 100}{\text{fMLP } 10^{-8}\text{ M abs.} - 0\%\ \text{release abs.}}$$

The chemotaxis inhibition rate of each 14-membered ring macrolide compound is shown in Fig. 2.

Although EM, CAM and RXM have been reported to

Fig. 2. Effects of EM, CAM, RXM and EM derivatives on rat leucocyte chemotaxis.

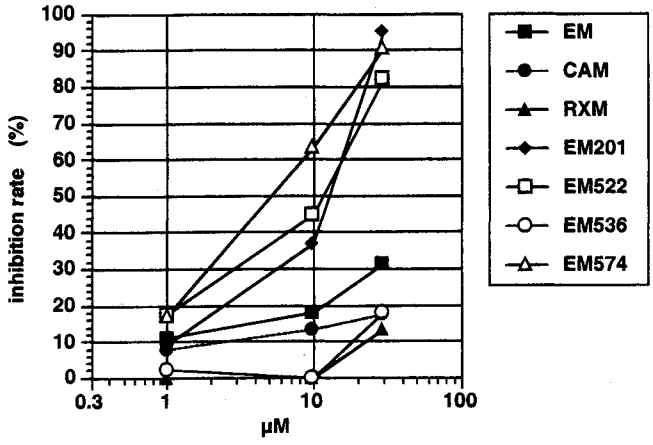


Table 1. Biological activities of EM, CM, RXM and EM derivatives.

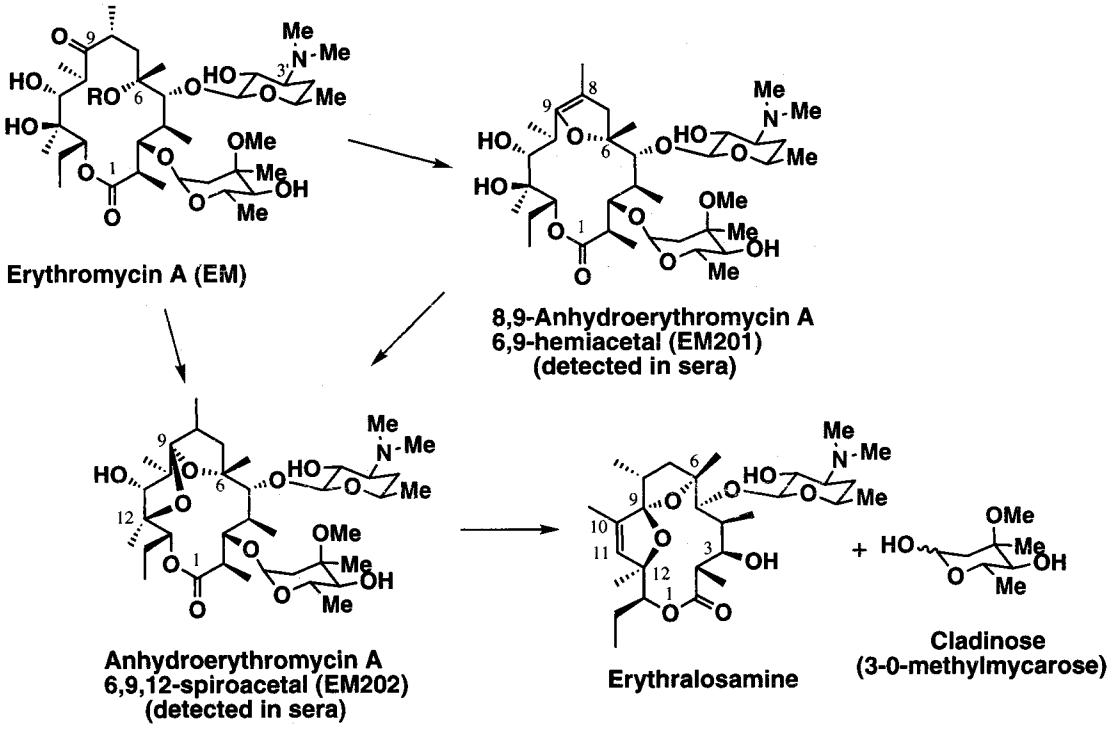
	Chemotaxis (inhibition:%)			Antimicrobial activity[a] (MIC; BS[b])	GMS activity[ac]
	1μM	10μM	30μM	(μg/ml)	(EM = 1)
EM	11.2	18.0	31.4	0.1	1
CAM	7.9	13.4	17.7	0.1	0.2
RXM	0.0	0.0	13.5	0.4	not tested
EM201	9.2	36.9	95.3	25	10
EM522	17.7	44.9	82.4	>100	15
EM536	2.2	0.0	18.1	>100	1986
EM574	17.0	63.3	90.7	>100	248

a ŌMURA, S. et al. ; J. Antibiotics 38 : 1631, 1985

b BS: Bacillus subtilis ATCC 6633

c GMS activity : Gastrointestinal motor stimulating activity

Fig. 3. Intramolecular cyclization of erythromycin A *in vivo*.



have clinically inhibitory effects on chronic inflammatory airway disease, their inhibitory activity on chemotaxis was very weak even at the concentration of 30 μM.

However, the EM-derivatives except for EM536 exhibited 36~63% of inhibition on leucocyte chemotaxis. Thus, it was suggested that the new derivatives of 14-membered ring macrolides have strong anti-inflammatory effects at lower dosages than conventional macrolides. Their inhibition rates were over 80 % at a concentration of 30 μM.

The antimicrobial activity (MIC), GMS activity, and chemotaxis of EM, CAM, RXM, and EM derivatives are shown in Table 1. Comparing MIC with inhibition on chemotaxis, there were no relationships between them. Furthermore, the inhibitory activity on chemotaxis showed no relationship with GMS.

EM201 is an intermediary metabolite of EM[14,15]. EM522 and EM574 are derivatives of EM201 with modified dimethylamino groups. Thus, it might be supposed that EM exhibits strong anti-inflammatory activity when it is metabolized *in vivo* (Fig. 3).

References

1) HOMMA, H.: Definition of diffuse panbronchiolitis. J. Jpn. Soc. Intern. Med. 65: 649~659, 1976
2) HOMMA, H.; A. YAMANAKA, H. TANIMOTO, Y. CHIJIMATSU, S. KIRA & T. IZUMI: Diffuse panbronchiolitis: a disease of the transitional zone of the lung. Chest 83: 63~69, 1983
3) KUDOH, S.; T, UETAKE. K, HAGIWARA, M. HIRAYAMA, HUS LH, H. KIMURA & Y. SUGIYAMA: Clinical effect of low dose long term erythromycin chemotherapy on diffuse panbronchiolitis. Jap. J. Thorac. Dis. 25: 632~642, 1987
4) KADOTA, J.; O. SAKITO, S. KOHNO, H. SAWA, H. MUKAE, H. ODA, K. KAWAKAMI, K. FUKUSHIMA, K. HIRATANI & K. HARA: A mechanism of erythromycin treatment in patients with diffuse panbronchiolitis. Am. Rev. Respir. Dis. 147: 153~159, 1993
5) SUGIHARA, E: Effect of macrolide antibiotics on neutrophil function in human peripheral blood. Kansenshogaku Zasshi 71: 329~336, 1997
6) AZUMA, A.; T. FURUTA, T. ENOMOTO, Y. HASHIMOTO, K. UEMATSU, N. NUKARIYA, A. MURATA & S. KUDOH: Preventive effect of erythromycin on experimental bleomycin-induced acute lung injury in rats. Thorax 53: 186~189, 1998
7) ŌMURA, S.; K. TSUZUKI, T. SUNAZUKA, H. TOYODA, I. TAKAHASHI & Z. ITOH: Gastrointestinal motor-

stimulating activity of macrolide antibiotics and the structure-activity relationship. J. Antibiotics 38: 1631~1632, 1985

8) ŌMURA, S.; K. TSUZUKI, T. SUNAZUKA, S. MARUI, H. TOYODA, N. INATOMI & Z. ITOH: Macrolides with gastrointestinal motor stimulating activity. J. Med. Chem. 30: 1941~1943, 1987

9) TSUZUKI, K.; T. SUNAZUKA, S. MARUI, H. TOYODA & S. ŌMURA: Motilides, macrolides with gastrointestinal motor stimulating activity. I. *O*-Substituted and tertiary *N*-substituted derivatives of 8,9-anhydroerythromycin A 6,9-hemiacetal. Chem. Pharm. Bull. 37: 2687~2700, 1989

10) SUNAZUKA, T.; K. TSUZUKI, S. MARUI, H. TOYODA, S. ŌMURA, N. INATOMI & Z. ITOH: Motilides, macrolides with gastrointestinal motor stimulating activity. II. Quanternary *N*-substituted derivatives of 8,9-anhydroerythromycin A 6,9-hemiacetal and 9,9-dihydroerythromycin A 6,9-epoxide. Chem. Pharm. Bull. 37: 2701~2709, 1989

11) KONDO, Y.; K. TORII, S. MURA & Z. ITOH: Erythromycin and its derivatives with motilin-like biological activities inhibit the specific binding of 125I-motilin to duodenal muscle. Biochem. Biophys. Res. Commun. 150: 877~882, 1988

12) ŌMURA, S.; Y. KONDO & Z. ITOH: Motilide, motilin-like macrolide. *In* Motilin. *Ed.*, Z. ITOH, pp. 245~256, Academic Press, New York, 1990

13) SUNAZUKA, T.; H. TAKIZAWA, M. DESAKI, K. SUZUKI, R. OBATA, K. OTOGURO & S. ŌMURA: Effects of erythromycin and its derivatives on interleukin-8 release by human bronchial epithelial cell line BEAS-2B cells. J. Antibiotics 52: 71~74, 1999

14) KIRST, H. A. & G. D. SIDES: Metabolism of macrolides. *In* Macrolides. *Ed.*, A. J. BRYSKIER *et al.*, pp. 485~494, arnette blackwell, Paris, 1993

15) TSUJI, K.: Fluorimetric determination of erythromycin and erythromycin ethylsuccinate in serum by a high-performance liquid chromatographic post-column, on-stream derivatization and extraction method. J. Chromatogr. 158: 337~348, 1978