VOL. 52, NO. 1, JAN. 1999 THE JOURNAL OF ANTIBIOTICS pp. 71 — 74

COMMUNICATIONS TO THE EDITOR

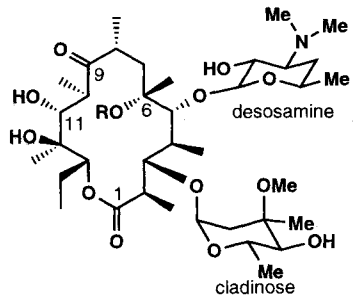
Effects of Erythromycin and Its Derivatives on Interleukin-8 Release by Human Bronchial Epithelial Cell Line BEAS-2B Cells

Sir:

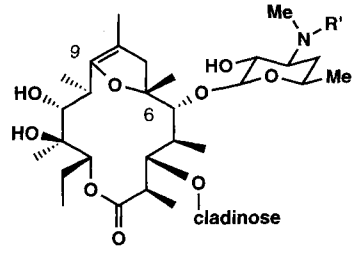
Macrolide antibiotics are widely used as antimicrobial agents. Previously, we discovered that erythromycin (EM) and its derivatives with no antimicrobial activity have strong gastrointestinal motor-stimulating (GMS) activity[1,2]. We proposed the generic name 'motilide' for a series of macrolides with motilin-agonistic activity[3,4]. About the same time, some macrolide antibiotics, especially EM-A and clarithromycin (CAM) have been reported to be effective against diffuse panbronchiolitis (DPB) which is one of chronic airway inflammatory diseases[5,6]. Although therapeutic mechanisms of these macrolides are not yet clear, it is considered that those responses are due to anti-inflammatory action rather than antimicrobial action[7]. Furthermore, we previously reported that EM-A and CAM suppressed mRNA levels as well as the release of pro-inflammatory cytokines, interleukin (IL) -6 and IL-8 in human bronchial epithelial cell line BEAS-2B (BEAS-2B cells) and primary normal bronchial epithelial cells[8,9]. We report here the suppressive effect of IL-8 release, the antimicrobial activity and GMS activity of EM-A, CAM and EM derivatives (Fig. 1) and describe the structure-activity relationships.

BEAS-2B cells[10] (a kind gift from Drs. J. F. LECHNER and C. C. HARRIS, National Cancer Institute, Bethesda, MA) were cultured by the method reported previously[8,9,11] with some modification. The cells were plated onto collagen coated 24-well culture plates at a density of 1×10^5 cells/well in hormonally defined Ham's F12 medium (HD-F12). The HD-F12 contained 1% penicillin-streptomycin, 5 μg/ml insulin, 5 μg/ml transferrin, 25 ng/ml epidermal growth factor, 15 μg/ml endothelial cell growth supplement, 2×10^{-10} M triiodothyronin and 10^{-7} M hydrocortisone. The cells were incubated at 37°C in 95% air-5% CO_2. Upon confluency, the cultured cells were washed with Hanks' balanced solution without calcium and magnesium, the media were replaced by fresh HD-F12, and 10^{-6} M macrolide anti-

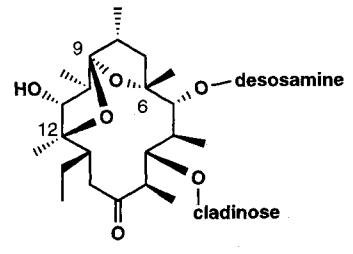
Fig. 1. Structures of erythromycin, its derivatives and clarithromycin.



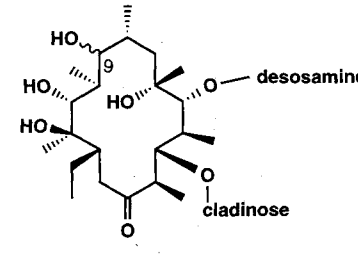
Erythromycin A (EM-A) R = H
Clarithromycin (CAM) R = Me



1 R' = Me
2 R' = H
3 R' = $CH(CH_3)_2$
4 R' = $(CH_2)_3CH_3$
5 R' = Ac
6 R' = Me, $-CH_2C{\equiv}CH{\bullet}Br$

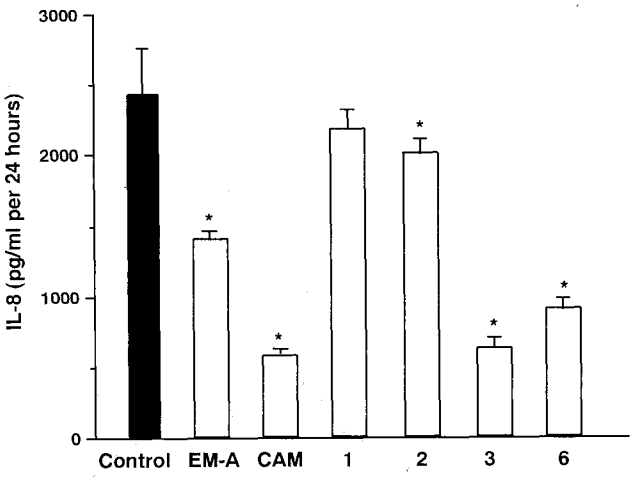


7



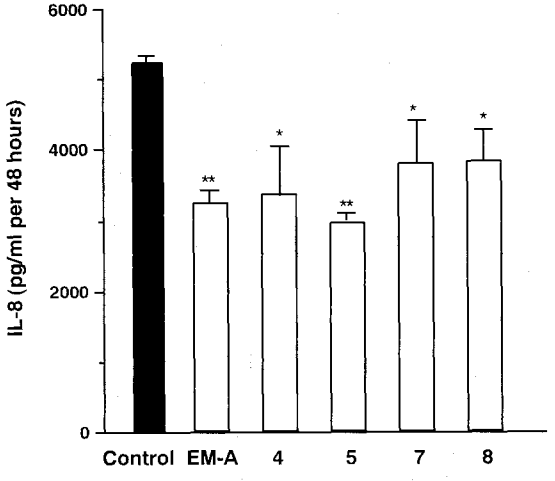
8

Fig. 2. Effects of erythromycin (EM), its (EM) derivatives and clarithromycin (CAM) on IL-8 release by BEAS-2B cells.



Each columnindicates the mean±S.E.M. * $P<0.05$ compared with the control group (Control) by ANOVA.

Fig. 3. Effects of erythromycin (EM) and its (EM) derivatives on IL-8 release by BEAS-2B cells.



Each column indicates the mean±S.E.M. * $P<0.05$, ** $P<0.01$ compared with the control group (Control) by ANOVA.

Table 1. Antimicrobial activities (MIC) and gastrointestinal motor stimulating (GMS) activities of erythromycin, its derivatives and clarithromycin.

Compound	Antimicrobial activity (MIC; μg/ml)[a]					GMS acitivity[a]
	SA[b]	BS	BC	EC	KP	
Erythromycin A (EM-A)	0.2	0.1	0.1	12.5	6.25	1[c]
Clarithromycin (CAM)	0.1	0.1	0.1	6.25	6.25	0.2
1	50	25	25	>100	>100	10
2	>100	>100	>100	>100	>100	14.9
3	>100	>100	>100	>100	>100	248
4	>100	>100	>100	>100	>100	8.4
5	>100	>100	>100	>100	>100	<1
6	100	100	100	>100	>100	2890
7	12.5	ND	6.25	>100	>100	3
8	6.25	3.13	3.13	>100	>100	0.7

[a] In the part of data, MIC and GMS activity of EM derivatives, were obtained from our previous reports[1,12,13].

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

[c] The activity of EM-A was taken to be 1.

biotics and their derivatives were added to each well, and incubated for 24 or 48 hours. Specific immunoreactivity for IL-8 in the cultured supernatants was measured by ELISA kits as described previously[9]. The results were analyzed by non-parametric equivalents of analysis of variance (ANOVA) for multiple comparison as reported[8,9,11]. Minimum inhibitory concentrations (MIC) of CAM and anhydro-erythromycin A (**7**) against test organisms were estimated by agar dilution method. GMS activities of CAM and **7** were carried out by the method described previously[1,2]. MIC and GMS activities of EM derivatives were obtained from our previous reports[1,12,13].

The results were shown in Figs. 2 and 3. EM-A exhibited similar suppressive effect on IL-8 release by BEAS-2B cells which were treated with 24 or 48 hours.

CAM, de(*N*-methyl)-*N*-isopropyl-8,9-anhydroerythromycin A 6,9-hemiacetal (**3**) and *N*-propargyl-8,9-anhydroerythromycin A 6,9-hemiacetal bromide (**6**) exhibited the strong suppressive effect on IL-8 release by the cells. EM-A, de(*N*-methyl)-*N*-butyl-anhydroerythromycin A 6,9-hemiacetal (**4**) and de(*N*-methyl)-*N*-acetyl-anhydroerythromycin A 6,9-hemiacetal (**5**) exhibited moderate suppressive effect on IL-8 release by the cells. **7** and 9-dihydro EM-A (**8**) exhibited weak effect on IL-8 release by the cells. 8,9-anhydroerythromycin A 6,9-hemiacetal (**1**) and de(*N*-methyl)-8,9-anhydroerythromycin A 6,9-hemiacetal (**2**) did not exhibit statistically significant effect on IL-8 release by the cells. Furthermore, we studied the effects of methymycin (12-membered macrolide), and oleandomycin, spiramycin, tylosin and rokitamycin (16-membered macrolides) in this system. But those macrolides did not exhibit statistically significant effect when added to the cells at 10^{-6} M (data no shown). Our findings suggest that 14-membered macrolide such as EM had specifically suppressive effect on the release of cytokine such as IL-8 from bronchial epithelial cells.

The antimicrobial and GMS activities of EM derivatives were shown in Table 1 in comparison with these activities and the suppressive effect of EM derivatives on IL-8 release. There are no relationship among the suppressive effect of IL-8 release, MIC and GMS activities. Among derivatives, compound **5** is the most interesting, because this compound showed the moderate suppressive effect of IL-8 release, but no antimicrobial and GMS activities.

We are further investigating for a possible development of a new type anti-inflammatory agent in EM derivatives.

Acknowledgment

We wish to thank Drs. I. TAKAHASHI and Z. ITOH, Gunma University, for the GMS assay, and Dr. R. MASUMA, the Kitasato Institute, for the MIC assay.

TOSHIAKI SUNAZUKA
HAJIME TAKIZAWA†
MASASHI DESAKI†
KUNIHIKO SUZUKI
RIKA OBATA
KAZUHIKO OTOGURO
SATOSHI ŌMURA*

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

† Department of Medicine and Physical Therapy,
University of Tokyo, School of Medicine,
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

(Received September 21, 1998)

References

1) 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
2) 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
3) KONDO, Y.; K. TORII, S. ŌMURA & 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
4) ŌMURA, S.; Y. KONDO & Z. ITOH: Motilide, motilin-like macrolide. *In* Motilin. *Ed.*, Z. ITOH, pp. 245～256, Academic Press, New York, 1990
5) KUDOH, S.; T. UETAKE, K. HAGIWARA, M. HIRAYAMA, L.-H. HUS, H. KIMURA & Y. SUGIYAMA: Clinical effect of low-dose, long-term erythromycin chemotherapy on diffuse panbronchiolitis. Jpn. J. Thorac. Dis. 25: 632～642, 1987
6) TAKEDA, H.; H. MIURA, M. KAWAHIRA, H. KOBAYASHI, S. OTOMO & S. NAKAIKE: Long-term administration study on TE-031 (A-56268) in the treatment of diffuse panbronchiolitis. Kansenshogaku Zasshi 63: 71～78, 1989
7) MIYATAKE, H.; F. TAKI, H. TANIGUCHI, R. SUZUKI, K. TAKAGI & T. SATAKE: Erythromycin reduces the severity of bronchial hyperresponsiveness in asthma. Chest 99: 670～673, 1991
8) TAKIZAWA, H.; M. DESAKI, T. OHTOSHI, T. KIKUTANI, H. OKAZAKI, M. SATO, N. AKIYAMA, S. SHOJI, K. HIRAMATSU & K. ITO: Erythromycin suppresses interleukin 6 expression by human bronchial epithelial cells. Biochem. Biophys. Res. Commun. 210: 781～786, 1995
9) TAKIZAWA, H.; T. OHTOSHI & K. ITO: Human bronchial epithelial cells produce cytokines relevant airway inflammation. ACI News 6: 146～150, 1994
10) REDDEL, R. R.; Y. KE, B. I. GERWIN, M. MCMENAMIN, J. F. LECHNER, R. T. SU, D. E. BRASH, J. B. PARK, J. S. RHIM & C. C. HARRIS: Transformation of human bronchial epithelial cells by infection with SV40 or adenovirus-12/SV 40 hybrid virus, or transfection via strontium phosphate coprecipitation with a plasmid containing SV40 early region genes. Cancer Res. 48: 1904～1909, 1988
11) TAKIZAWA, H.; T. OHTOSHI, K. OHTA, S. HIROHATA, M. YAMAGUCHI, N. SUZUKI, T. UEDA, A. ISHII, G. SHINDOH, T. OKA, K. HIRAMATSU & K. ITO: Interleukin 6/B cell stimulatory factor-2 is expressed and released by normal and transformed human bronchial epithelial cells. Biochem. Biophys. Res. Commun. 187: 569～602, 1992
12) TSUZUKI, K.; T. SUNAZUKA, S. MARUI, H. TOYODA, S. ŌMURA, N, INATOMI & Z. ITOH: 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

13) SUNAZUKA, T.; K. TSUZUKI, S. MARUI, H. TOYODA, S. ŌMURA, N, INATOMI & Z. ITOH: Motilides, macrolides with gastrointestinal motor stimulating activity. II. Quaternary *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