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NOTES

# Effect of 14-Membered Macrolide Compounds on Monocyte to Macrophage Differentiation

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Recently, it was reported that erythromycin (EM) and some other macrolide antibiotics showed efficacy on chronic inflammatory airway disease besides antibacterial activity, and the fact attracted attention.

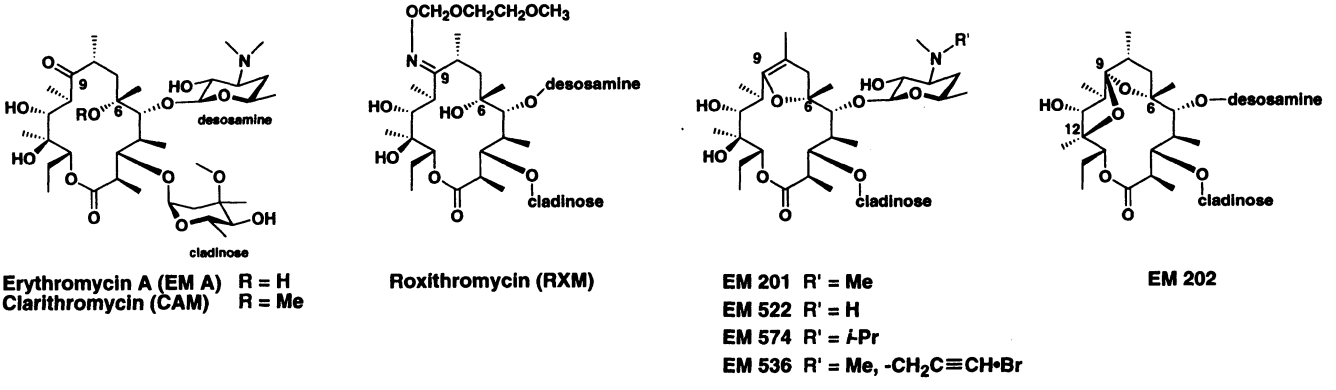
The prognosis for diffuses panbronchiolitis (DPB), one of incurable chronic inflammatory airway disease, was improved significantly by treatments with long term and low doses of such macrolide antibiotics[1,2]. Such a therapeutic efficacy is supposed to be caused by anti-inflammatory or immunomodulatory activity of macrolide antibiotics, and some basic researches were carried out on those activities.

EM and also azithromycin (AZM), one of 15-membered ring macrolide antibiotic, were showed to have inhibitory activity against the inflammatory functions of neutrophils *in vitro*[3]. The two antibiotics showed clinical effect in the treatment of DPB. EM exhibited also prophylactic effect on lung injury *in vivo* against bleomycin-induced acute lung injury model in rat[4].

We have recently reported on some non-antimicrobial activities of macrolides. That is, EM and its derivatives showed remarkable gastrointestinal motor-stimulating (GMS) activities[5,6] and the generic name 'motilide' was proposed for a series of macrolides having motilin-agonistic activity[7~10]. Furthermore, we clarified the suppressive effect of interleukin (IL)-8 release in human bronchial epithelial cell line by EM, clarithromycin (CAM) and eight other EM-derivatives[11]. IL-8 release was potently suppressed by CAM and two EM-derivatives that have no or weak antibacterial activities.

We also studied about the anti-inflammatory effect of EM, CAM, roxithromycin (RXM) and three non-antimicrobial EM-derivatives by examining their inhibitory activity against rats leucocytes chemotaxis[12]. The results indicated that structural factors to concern with antibacterial and anti-inflammatory activities are distinct. EM, CAM and RXM exhibited only weak chemotaxis inhibitory activities, while three non-antimicrobial EM-

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




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

**Roxithromycin (RXM)**

**EM 201 R' = Me**
**EM 522 R' = H**
**EM 574 R' = *i*-Pr**
**EM 536 R' = Me, -$CH_2C{\equiv}CH{\cdot}Br$**

**EM 202**

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derivatives showed remarkable activities.

On the other hand, some other interesting results were reported on immunomodulatory activities of CAM, josamycin and midecamycin acetate. These macrolides inhibited human T-cell function and IL-2 production by mitogens stimulation[13]. We have also reported that EM promoted monocyte to macrophage differentiation *in vitro*[14].

Thus, we supposed that non-antibacterial EM derivatives could have various immunopharmacological activities and that the study of the action mechanism should be useful for development of new therapeutic drugs of chronic airway disease. In this paper, we report on the immunomodulatory effect to promote human monocytic cell line THP-1 to macrophage shown by EM, CAM, RXM and five other EM-derivatives with no or weak antimicrobial activities (Fig. 1).

## Materials and Methods

Eight 14-membered macrolide compounds, erythromycin (EM), clarithromycin (CAM), roxithromycin (RXM), 8,9-anhydroerythromycin A 6,9-hemiacetal (EM201), anhydroerythromycin A 6,9,12-spiroacetal (EM202), de(*N*-methyl)-8,9-anhydroerythromycin A 6,9-hemiacetal (EM522), 8,9-anhydroerythromycin A 6,9-hemiacetal propargyl bromide (EM536), and de(*N*-methyl)-*N*-isopropyl-8,9-anhydroerythromycin A 6,9-hemiacetal (EM574), were chemically synthesized in our laboratory. They were dissolved in ethanol at 6~20 $\mu$M concentrations. The final ethanol concentrations were adjusted to 0.5% to avoid cells damage.

The promotive activities were determined by modifing the method of KEICHO *et al.* (1994)[14]. THP-1 cell line, derived from a patient with monocytic leukemia, was supplied by Japanese Cancer Research Resources Bank (Tokyo, Japan. Japan Health Sciences Foundation, at present).

THP-1 cells ($1\times10^5$ per well in 0.5 ml) were poured into 48-well tissue culture microplate (IWAKI, Japan) and cultured in the presence of phorbol myristate acetate (PMA; 2 ng/ml) or each macrolide compound (1~100 $\mu$M) alone or both for 4 days at 37°C under 5% $CO_2$ in 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) assay at 550 nm.

Fig. 2. Effects of EM, CAM, RXM and EM derivatives on THP-1 cells to macrophage differentiation.

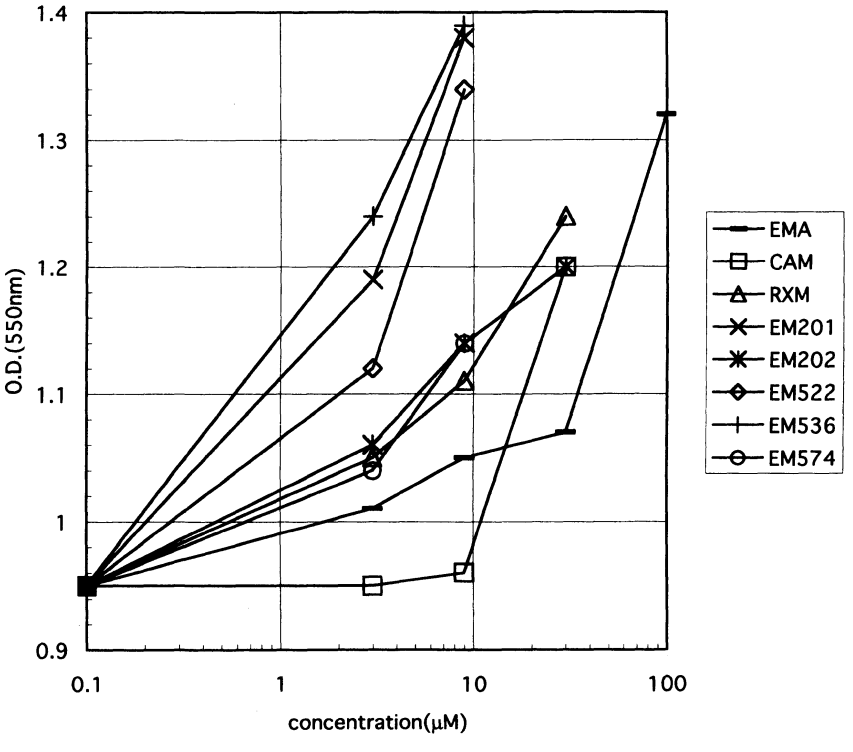

Table 1. Biological activities of EM, CAM, RXM and EM derivatives.

| | THP-1/Mϕ differentiation (promotion rank[a]) | | | Antimicrobial activity[b] (MIC; BS[c]) | GMS activity[b,d] | IL-8 release[e] (inhibition:%) | Chemotaxis[f] (inhibition:%) |
|---|---|---|---|---|---|---|---|
| | 3μM | 10μM | 30μM | (μg/ml) | (EM=1) | (1μM) | (30μM) |
| EM | − | ± | ± | 0.1 | 1 | 42.3 | 31.4 |
| CAM | − | − | + | 0.1 | 0.2 | 76.6 | 17.7 |
| RXM | − | ± | + | 0.4 | not tested | not tested | 13.5 |
| EM201 | + | ++ | not tested | 25 | 10 | 10.2 | 95.3 |
| EM202 | − | + | + | 6.3 | 3 | 27.8 | not tested |
| EM522 | ± | ++ | not tested | >100 | 15 | 17.1 | 82.4 |
| EM536 | + | ++ | not tested | >100 | 2890 | 62.2 | 18.1 |
| EM574 | − | ± | not tested | >100 | 248 | 74.4 | 90.7 |

a The each activity was indicated in four ranks which classified by the ratio to 100μM EM activity
−: 0~25%, ±: 25~50%, +: 50~100%, ++: >100%
b In the part of data, MIC and GMS activities were obtained from our previous reports
c BS: *Bacillus subtilis* ATCC 6633
d GMS activity: gastorointestinal motor stimulating activity
e In the part of data, inhibitory activity on IL-8 release were calculated by using of the data obtained from our previous report
f In the part of data, inhibitory activity on leucocytes chemotaxis were obtained from our previous reports

## Result and Discussion

In our previous observation, human monocytes differentiation was markedly promoted by EM at the concentration of 100 μg/ml[14]. Though THP-1 cells were not stimulated by EM alone, EM with a low dose of PMA showed significant increase of adherent cells. Therefore we studied THP-1 cell differentiation activities of the other macrolide compounds.

In the MTT assay, coapplication of 100 μM EM and 10 μM PMA exhibited 1.2~1.5 times of promotive activity relative to PMA alone (Fig. 2). The activities of the other macrolides were compared relative to that of 100 μM EM and the results are summarized in Table 1.

EM, CAM and RXM reported to have clinical promotive effect against chronic inflammatory airway disease showed promotive activity at the concentration of 30 μM, but they showed weak or no activity at 10 μM. In contrast, three EM-derivatives, EM201, EM522 and EM536, exhibited promotive effects on THP-1 cells differentiation even at 10 μM. Their activities were the same or more than that of EM at 100 μM concentration. Thus, it was suggested that these non-antimicrobial EM-derivatives have more potent immunomodulatory effect at lower dosages than conventional antimicrobial macrolides.

Their antimicrobial activities (MIC), gastrointestinal motor-stimulating (GMS) activities, IL-8 release inhibitory activities, and chemotaxis were also shown in Table 1. In comparison of their MIC with differentiation promotion activities, no proportional relationship was observed. The monocytes differentiation activity was also not related to any of GMS, IL-8 release inhibition or chemotaxis inhibition. Therefore, the monocyte differentiation promotive effect seemed to be independent from the other four activities.

EM201 is an intermediate metabolite of EM[15,16]. EM201 is produced from EM under acidic condition and quickly metabolized to EM202. EM522, EM536 and EM574 are derivatives of EM201 and their carbon skeletons are the same. Thus, it is presumed that EM gains immunomodulatory activity during its metabolism *in vivo*, and is further converted quickly to other structures having weak immunomodulatory activity. Further studies on EM201 derivatives are expected to result some desirable compounds having potent immunomodulatory activity.

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