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Direct comparison of anti-inflammatory effects of 14-, 15-, and 16-membered macrolide antibiotics in experimental inflammation model induced by carrageenan in rats

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Some macrolide antibiotics, which share a basic lactone ring structure, also exhibit anti-inflammatory actions in addition to their antibacterial activities. However, no study has directly compared anti-inflammatory effects on acute inflammation among macrolide antibiotics with the distinct size of the lactone ring. In this study, we evaluated and compared the anti-inflammatory activities of four 14-membered macrolides (erythromycin, clarithromycin, roxithromycin, oleandomycin), one 15-membered macrolide (azithromycin), and three 16-membered macrolides (midecamycin, josamycin, leucomycin) using a rat carrageenan-induced footpad edema model. All macrolide antibiotics were intraperitoneally administered to rats one hour before the induction of inflammatory edema with 1% λ-carrageenan. The anti-inflammatory effects on acute inflammation were evaluated by changing the edema volume. All 14-membered and 15-membered macrolide antibiotics significantly suppressed the development of edema. Conversely, none of the 16-membered macrolide antibiotics inhibited the growth of edema. In conclusion, compared to 16-membered macrolide antibiotics, 14-membered and 15-membered macrolide antibiotics have stronger anti-inflammatory effects. Further research should be done to determine why different lactone ring sizes should have distinct anti-inflammatory effects.

1. Introduction

The basic structure of macrolide antibiotics is a lactone ring with methyl side chain; they can be classified as 14-membered, 15-membered, 16-membered, and 18-membered macrolides. Many macrolide antibiotics have been produced thus far after erythromycin was first isolated from *Saccharopolyspora erythrea*. For more than half a century, it has been shown that many macrolide antibiotics have anti-inflammatory properties in addition to their antibacterial properties. First off, in the 1970s, Itkin and Menzel reported that erythromycin was clinically shown to reduce the severity of asthma (Itkin and Menzel 1970). Based on the fact that erythromycin has anti-inflammatory activity, basic research and clinical trials during and after the development of new clinically available macrolide antibiotics, including clarithromycin, roxithromycin, and azithromycin, have also investigated the anti-inflammatory properties of these drugs *in vivo*/*in vitro* (Zarogoulidis et al. 2012). Nonetheless, no study has directly contrasted the anti-inflammatory effects of macrolide antibiotics with regard to acute inflammation according to the lactone ring size of macrolide antibiotics.

A carrageenan-induced footpad edema model is an acute inflammation animal model well established by Van Arman et al. (1965). This acute inflammation model can evaluate the anti-inflammatory actions of agents by determining their inhibitory effects on edema volume. Even though it has past more than half a century since this model was established, it remains to be the standard model to investigate the anti-inflammatory actions of agents (Alnusaire et al. 2023; Duarah et al. 2023; Ullah et al. 2023).

In the present study, we used an experimental carrageenan-induced inflammation rats model to examine and directly compared the anti-inflammatory action of four 14-membered macrolides (erythromycin, clarithromycin, roxithromycin, oleandomycin: Fig. 1A), one 15-membered macrolide (azithromycin: Fig. 1B), and three 16-membered macrolides (midecamycin, josamycin, leucomycin:

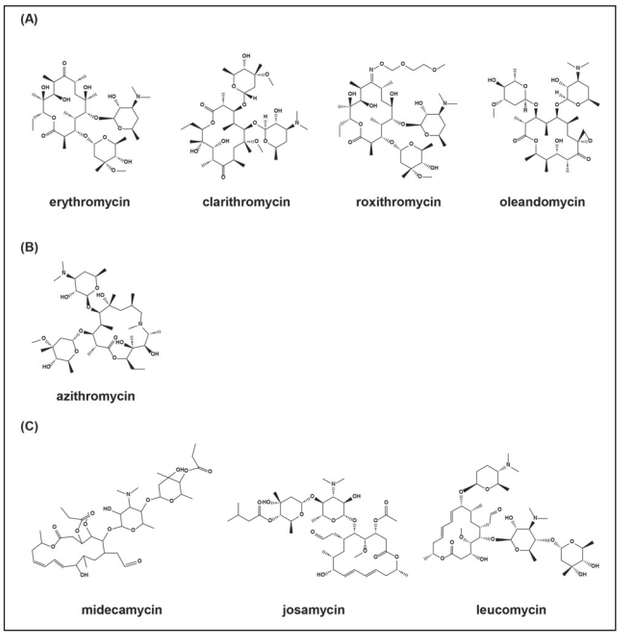

Fig. 1: Chemical structure of (A) 14-membered macrolide antibiotics, (B) 15-membered macrolide antibiotics, and (C) 16-membered macrolide antibiotics used in this study.

Fig. 1C) against acute inflammation. Due to its oral formulation, fidaxomicin, an 18-membered macrolide for the treatment of *Clostridioides difficile* (Tashiro et al. 2022), was excluded from the evaluation of anti-inflammatory actions in this study.

2. Investigations, results and discussion

2.1. Determination of evaluation time to compare the anti-inflammatory action of macrolide antibiotics

We first evaluated the changes in time-course edema volume after the administration of carrageenan to rats in order to determine the assessment period for the anti-inflammation effect test. Consequently, the edema volume peaked three hours after the administration of carrageenan and then gradually reduced (Fig. 2). However, at all times that were noted, erythromycin treatment (100 mg/kg) inhibited the production of edema (Fig. 2). It is well known that carrageenan induces edema in two-steps: histamine and serotonin cause the first inflammation to occur 0.5 to 1 hour (early phase) after the carrageenan is administered, and the second inflammation is induced 2 to 3 hour (late phase) after the carrageenan administration by prostaglandins, leukotrienes, and bradykinin (Morris 2003). To observe the effect of macrolide antibiotics on inflammations induced by factors in both the early and late phases, we decided to evaluate the anti-inflammatory actions of macrolide antibiotics three hours after carrageenan administration in the subsequent investigations.

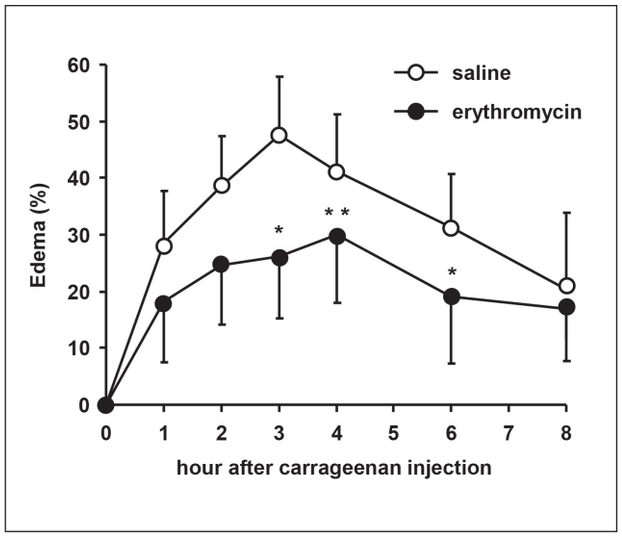

Fig. 2: Time-course of the edema rate changes after saline and erythromycin administration. Mean±standard deviation (n=7-11). * p<0.05, ** p<0.01

2.2. Determination of the dose to compare the anti-inflammatory action of macrolide antibiotics

To decide the dose for the evaluation of the anti-inflammation effect, we evaluated the dose-dependent inhibitory effects of edema using erythromycin as a representative macrolide antibiotic. Similar to the previous finding reported by Ianaro et al. (2000), the edema volume three hours after the carrageenan administration was dose-dependently decreased as the dose of erythromycin increased (Fig. 3). Significant suppression was observed at erythromycin doses of 25, 50, and 100 mg/kg (Fig. 3). Based on these findings, we chose to compare the anti-inflammatory actions of macrolide antibiotics at a single dose of 100 mg/kg in carrageenan-induced footpad edema model rats.

2.3. Comparison of the anti-inflammatory effects of each macrolide antibiotic on acute inflammation

We compared the inhibitory effects of four 14-membered macrolides (erythromycin, clarithromycin, roxithromycin, oleandomycin), one 15-membered macrolide (azithromycin), and three 16-membered macrolides (midecamycin, josamycin, leucomycin) at 100 mg/kg on edema. Previous studies showed that the increase in edema brought on by the administration of carrageenan was decreased by the oral administration of roxithromycin, clarithromycin, azithromycin, and erythromycin (Agen et al. 1993; Ianaro et al. 2000; Scaglione and Rossoni 1998). In addition to these macrolide antibiotics, this study first revealed that oleandomycin, a 14-membered macrolide, exhibited inhibitory effects on edema 3 hours after the carrageenan administration (Fig. 4). Furthermore, the strength of anti-inflammatory activities of both the 14-membered macrolide and 15-membered macrolides were almost the same. These results indicated that both 14-membered and 15-membered macrolides had anti-inflammatory properties when applied to acute inflammation. However, none of the 16-membered macrolides showed any discernible inhibitory impact (Fig. 4). Not limited to carrageenan-induced acute inflammation, to the best of our knowledge, there is no information available on the anti-inflammatory properties of 16-membered macrolide. It follows that 16-membered macrolide antibiotics would be less effective against inflammation than 14- and 15-membered macrolide antibiotics. It is unclear why the anti-inflammatory properties were different among macrolide antibiotics, but the different pharmacokinetic properties, such as plasma retention and distribution at the site of inflammation, may be related.

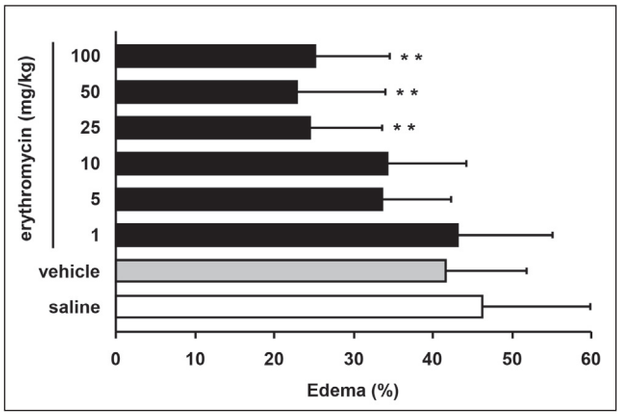

Fig. 3: Dose-dependent anti-inflammatory effect of erythromycin. The edema rates were determined at 3 hours after carrageenan administration. Mean ± standard deviation (n=7-12). ** p<0.01

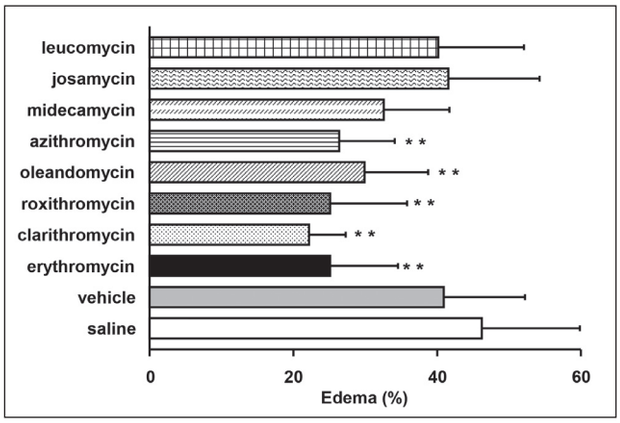

Fig. 4: The anti-inflammatory effects of macrolide antibiotics. All macrolide antibiotics were administered at a dose of 100 mg/kg. The edema rates were determined at 3 hours after carrageenan administration. Mean±standard deviation (n=8-10). ** p<0.01

2.3. Conclusion

Without a doubt, certain macrolide antibiotics possess anti-inflammatory activities in addition to their antibiotic effects. Nevertheless,

the extent to which macrolide antibiotics have anti-inflammatory properties remains unclear at this time. The results of this study showed that 14-membered and 15-membered macrolide antibiotics possess similar anti-inflammatory activity, but 16-membered macrolide antibiotics do not show anti-inflammatory activity under the same experimental conditions. Thus, when anti-inflammatory activities are needed in addition to an antibacterial effect, selecting either 14-membered or 15-membered macrolide antibiotics may be preferable to 16-membered macrolide antibiotics. Furthermore, there are attempts to develop anti-inflammatory agents derived from macrolide structures (lactone ring) (Sadamatsu et al. 2020; Yang et al. 2023), indicating that the results obtained in this study may have been useful in the design of a macrolide-based anti-inflammatory agent. However, we did not evaluate the anti-inflammatory activity of all macrolide antibiotics. To fully determine the anti-inflammatory effects of macrolide antibiotics based on the size of the lactone ring, additional research is necessary to clarify the anti-inflammatory activity of other macrolide antibiotics for acute inflammation.

3. Experimental

3.1. Macrolide antibiotics

Erythromycin and DMSO were purchased from Nacalai Tesque Inc., clarithromycin was purchased from Dainabot CO., LTD, roxithromycin, oleandomycin, and midecamycin were purchased from Sigma-Aldrich Japan, azithromycin was purchased from LKT Labs, josamycin and leunomycin were purchased from Wako Pure Chemical Industries. All macrolide antibiotics were dissolved in DMSO before use.

4.2. Laboratory animals

Wistar rats (male, 4-week-old) were purchased from Sankyo Labo Service Corporation and used for experiments after 1-week acclimation. The animal experiments were performed according to ARRIVE guidelines in this study. All animal experiments were approved by the Animal Care and Use Committee of Keio University (approval #: 14021-(0)).

4.3. Evaluation of the anti-inflammatory effects

Either saline, vehicle (DMSO), or macrolide antibiotics solution was intraperitoneally injected into rats at a volume of 0.2 mL/kg. At 1 hour after each test sample administration, 0.1 mL of 1% λ-carrageenan in physiological saline (Sigma-Aldrich Japan) was subcutaneously injected into the footpad of the rat right posterior limb as the same procedures in our previous study (Isobe et al. 2023; Ito et al. 2023). At predetermined times, the foot volume of the right posterior limb was measured using a foot volume-measuring device (Plethysmometer, UNICOM Co., Ltd.). The edema rate was calculated using the following formula:

$$\text{Edema rate (\%)} = \frac{Vt - Vc}{Vc} \times 100$$

Vc: Foot volume before carrageenan treatment (mL)
Vt: Foot volume t hours after carrageenan treatment (mL)

4.4. Statistical analysis

All data were statistically analyzed by Tukey test using SPSS® 22 for Windows software. Differences were considered statistically significant when the p-value was <0.05.

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Conflict of interest: none declared

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