Comparative anti-inflammatory effects of roxithromycin, azithromycin and clarithromycin

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There are many published reports on the anti-inflammatory effects of macrolides, some dating back to the introduction of erythromycin. Macrolides have been shown to affect a number of the processes involved in inflammation, including the migration of neutrophils, the oxidative burst in phagocytes and the production of various cytokines, although the precise mechanisms are not clear. These effects have been linked to the ability of macrolides to accumulate in mammalian cells. Roxithromycin, a macrolide with better plasma concentrations and higher tissue concentrations than erythromycin, has been tested in a standard animal model used for evaluating anti-inflammatory drugs. When rats were given a prophylactic dose (20 mg/kg), roxithromycin suppressed the oedema produced by injecting carrageenin into the paw with effects almost equal to that seen with the non-steroidal anti-inflammatory drug nimesulide. Azithromycin and clarithromycin, macrolides with better pharmacokinetics than erythromycin, only showed slight anti-inflammatory effects. These results confirm that roxithromycin has anti-inflammatory properties in vivo and encourage the investigation of its mode of action.

Introduction

There have been numerous reports over the past 25 years of macrolide antibiotics having anti-inflammatory activity. They were shown to be effective in alleviating asthma as long ago as 1970 and 1974. More recently, Miyatake et al. have shown that erythromycin had a favourable effect in patients with bronchial asthma, while others have used erythromycin and the newer macrolides in cases of diffuse panbronchiolitis (DPB).

Erythromycin has been used with some success for treating acne and is active in various animal tests of inflammatory cutaneous conditions. There are numerous reports of macrolides affecting the migration of neutrophils and the production of cytokines, although these are often contradictory.

Although the precise mechanisms of these anti-inflammatory effects are not clear, a number of authors have suggested that, in some conditions, interactions between macrolides and white blood cells are important. The ability of macrolides to accumulate in mammalian cells may be important in reducing inflammation. Three new macrolide derivatives, azithromycin, clarithromycin and roxithromycin, which have more favourable pharmacokinetic profiles and better cell penetration than erythromycin, have been tested in a rat paw oedema model. Their ability, when administered prophylactically, to prevent the inflammatory response to carrageenin was compared with that of nimesulide, a new non-steroidal anti-inflammatory drug (NSAID).

Materials and methods

Rat carrageenin paw oedema

The rat carrageenin paw oedema model as described by Van Arman et al. was used. Groups of five male Wistar rats weighing 200–250 g (Charles River, Calco, Italy) were given water and then an oral dose of a macrolide, nimesulide or saline. Hind paw volumes were measured by mercury plethysmography 1 h later. After baseline measurements of paw volume, 0.05 mL of a 1% (w/v) solution of carrageenin in saline was injected into the plantar region of the right hind paw. The paw volumes were measured at hourly intervals up to 6 h. The change in paw volume was calculated for each time point.

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Compounds

Three macrolides, namely azithromycin (Pfizer, Rome, Italy), clarithromycin (Abbott, Rome, Italy) and roxithromycin (Hoechst Marion Roussel, Milan, Italy), were used at a single oral dose of 20 mg/kg. The anti-inflammatory drug nimesulide (Helsinn, Biasca, Switzerland) was used as control at a single oral dose of 4 mg/kg. All drugs were administered 1 h before injecting carrageenin into the paw. Carrageenin (Sigma, St Louis, MO, USA) was prepared as a 1% (w/v) solution in saline.

Statistical analysis

The significance of differences between control and treated groups was evaluated by one-way analysis of variance (ANOVA) followed by Student Newman–Keuls test. P values of <0.05 were considered significant.

Results

The change in mean paw volume for the various groups is shown in Figure 1. The baseline measurements for the various groups were very similar, with mean values of 1.34–1.38 mL. In the untreated control animals, swelling of the paw was evident by 1 h after administration of carrageenin and reached a peak at 4 h, with an increased volume of 0.83 mL, decreasing slowly thereafter. The NSAID nimesulide suppressed this swelling almost completely, the change in volume being approximately 0.1 mL. Compared with the control, azithromycin and clarithromycin slightly reduced the swelling (giving volumes of 0.56 and 0.49 mL, respectively), while roxithromycin reduced it markedly (giving a volume of 0.25 mL), being only slightly less effective than nimesulide.

In all groups the maximum swelling was seen 4 h after administration of carrageenin; the mean increases in paw volumes at 4 h are presented in Figure 2. The increase in paw volume in the group receiving roxithromycin was 0.25 mL, which compares favourably with that found for nimesulide (0.13 mL). These values both differ significantly from that of the control group (P < 0.001). The maximum increase with azithromycin and clarithromycin was 0.56 and 0.49 mL, respectively; again each of these values is significantly different from the paw volume attained with the saline control (P < 0.05).

Discussion

The anti-inflammatory properties of macrolides were first recognized in the early 1970s, when erythromycin and troleandomycin, for example, were shown to be effective in the treatment of patients with asthma.1,2 More recently, erythromycin has been found to be of benefit in patients with bronchial asthma.3,4 In Japan, erythromycin, clarithromycin and roxithromycin have all been shown to be effective in the treatment of patients suffering from DPB, a chronic inflammation of the bronchioles which is more prevalent in Japan than elsewhere.5,6 Shirai et al.5 found that roxithromycin was more effective than clarithromycin or erythromycin in the treatment of DPB. The authors considered that these effects were not related to interactions with polymorphonuclear lymphocytes (PMNs); however, they used cells collected from normal healthy volunteers and not patients. Kadota et al.5 harvested neutrophils from patients treated with roxithromycin, and found a reduction in both the interleukin concentrations produced and the number of neutrophils in bronchial alveolar lavage (BAL) fluid.

These two studies illustrate some of the problems in
understanding the mechanisms responsible for the anti-inflammatory properties of macrolides. Many of the more detailed studies that have addressed this area have given contradictory results. For example, some authors have found that erythromycin inhibits the chemotaxis of PMNs, while others have found that it does not. These differences may be explained, in part, by technical differences, as some tests were carried out in animals, some used cells harvested from animals treated with the macrolide (ex vivo) and some were conducted purely in vitro. In addition, the mechanism by which conventional anti-inflammatory drugs work is complex and not fully understood.

The ability of macrolides to accumulate in mammalian cells may play an important role in their anti-inflammatory activity, as PMNs, although important in antimicrobial defence, also contribute to inflammation and tissue damage. An excessive influx of PMNs produces high localized levels of oxidants and proteolytic enzymes. Macrolides, including roxithromycin, reduce the oxidative burst in phagocytic cells and increase intracellular levels of cAMP, contributing to an anti-inflammatory effect.

Other important aspects of the inflammatory response include the production of cytokines, and macrolides have been reported to influence the levels of a number of inflammatory leukotrienes. Roxithromycin, for example, has been shown to inhibit the production of interleukin 1β and tumour necrosis factor α (TNF-α) in human peripheral blood monocytes in a dose-dependent manner. In a series of experiments using mice dosed with roxithromycin and mitogen-activated human peripheral leucocytes, Konno et al. showed that roxithromycin had a dose-dependent inhibitory effect on the production of a number of interleukins and TNF-α. They concluded that these effects would contribute to anti-inflammatory and anti-asthmatic activity.

Conventional anti-inflammatory drugs are tested in a range of animal models designed to demonstrate a direct overall anti-inflammatory effect. Many rely on the injection of an irritant or inflammatory substance, such as croton oil, carrageenin, poly-L-arginine or lipopolysaccharide. The carrageenin rat paw oedema model, described by Van Arman et al., is well established and widely used to demonstrate the effects of anti-inflammatory agents. It remains a standard model for new agents; Brand et al. used it in their evaluation of capsaicin analogues, while Kumakura et al. used it to demonstrate the effects of a prodrug of indomethacin.

In the study reported here, all three macrolides tested were able to reduce the inflammation provoked by carrageenin in the rat paw model. Roxithromycin reduced the inflammation more than either azithromycin or clarithromycin, almost completely suppressing the oedema produced by carrageenin. Roxithromycin was only slightly less active than nimesulide. These results confirm the findings of Agen et al., who found that roxithromycin had only slightly less anti-inflammatory activity than the NSAID indomethacin. In a poly-L-arginine rat paw model, roxithromycin had greater anti-inflammatory activity, equal to that of indomethacin, but it was less active than indomethacin in a chronic granuloma produced by implanting polyester sponges intraperitoneally. The authors suggest that the activity of roxithromycin is not related to inhibition of prostaglandin synthesis. We have investigated this by testing roxithromycin in another inflammatory model in rats, using implanted sponges soaked in carrageenin (unpublished data). In contrast with the previous authors, we found that, although the numbers of polymorphs and the levels of leukotriene B4 were little affected, marked dose-dependent inhibition of prostaglandin E2 and thromboxane B2 occurred. A decrease in the volume of exudate was also produced.

Individual NSAIDs act on separate parts of the inflammatory cascade; for example, indomethacin and nimesulide reduce the production of prostaglandins by inhibiting cyclooxygenase, while others inhibit 5-lipoxygenase, an enzyme that is important in leukotriene biosynthesis. These different modes of action lead to different responses in various models of inflammation, and the results of such studies require careful analysis.

The clinical reports of the beneficial effects of roxithromycin in inflammatory conditions, taken together with the results reported here, confirm the marked anti-inflammatory activity of roxithromycin. Although the anti-inflammatory activity of roxithromycin is secondary to its anti-infective capacity, these results suggest that further work should be conducted to investigate the mechanism of these effects.

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References

on cytokine. (In Japanese) Kansenshogaku Zasshi (Journal of the Japanese Association for Infectious Diseases) 68, 27–33.


