Suppression of matrix metalloproteinase production from nasal fibroblasts by macrolide antibiotics *in vitro*

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ABSTRACT: It is well known that low-dose and long-term administration of macrolide antibiotics favourably modify the clinical status of chronic airway inflammatory diseases. However, the therapeutic mode of action of macrolide antibiotics is not well understood. The present study aimed to examine the influence of macrolide antibiotics, roxithromycin (RXM) and josamycin (JM) on matrix metalloproteinase (MMP) production from nasal polyp fibroblasts (NPF) *in vitro*.

NPF, at a concentration of 2.5×10^5 cells·mL^{-1}, were stimulated with tumour necrosis factor (TNF)-α in the presence of various concentrations of RXM or JM for 24 h. MMP-2 and -9 levels in culture supernatants were analysed by ELISA, and MMP mRNA expression was examined by RT-PCR. The influence of RXM on nuclear factor (NF)-κB and activator protein (AP)-1 activation was also examined.

Addition of RXM (but not JM) at 5.0 and 7.5 µg·mL^{-1} significantly suppressed the production of MMP-2 and -9 from NPF induced by TNF-α stimulation. RXM also suppressed MMP mRNA expression through the inhibition of NF-κB and AP-1 activation.

The present results suggest that the suppressive activity of roxithromycin on MMP-2 and -9 production is, in part, responsible for the therapeutic action of macrolides on chronic airway inflammatory diseases.
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It is well accepted that sinobronchial syndrome involves the coexistence of chronic rhinosinusitis and chronic lower airway inflammation, *i.e.* chronic bronchitis and diffuse panbronchiolitis (DPB). Although these diseases are resistant to several types of treatment, low-dose and long-term administration of 14-membered macrolide antibiotics, such as erythromycin and roxithromycin (RXM), can improve the clinical condition of these diseases [1–3]. Recently, long-term use of azithromycin, a newly developed 16-membered macrolide antibiotic, has been reported to be able to favourably modify lung function of patients with cystic fibrosis (CF) [4, 5]. These reports clearly indicate that the prognosis of these life-threatening airway diseases, especially DPB and CF, may improve dramatically, but the mode of action of this macrolide therapy is not well understood.

Since low-dose, long-term erythromycin treatment, which is below the minimum concentration (200–600 mg·day^{-1}) required for killing common superinfecting organisms, has been reported to be effective against DPB and chronic sinusitis [1–3], the therapeutic mode of action of macrolides is generally not believed to be due to their anti-bacterial effects. *In vitro* studies clearly show that erythromycin strongly suppresses chemotaxis and the production of oxygen free radicals (O_2 and H_2O_2, *etc.*) by polymorphonuclear leukocytes when the cells were cultured in the presence of erythromycin [6, 7]. Previous work by the current authors has also shown the suppressive activity of RXM on the production of pro-inflammatory cytokines, such as interleukin (IL)-1 and tumour necrosis factor (TNF)-α, from both human peripheral blood monocytes and mast cells *in vitro* [8, 9]. The suppressive activity of RXM on inflammatory cytokine production has also been observed *in vivo*: treatment of mice with RXM once a day for >3 weeks markedly suppressed the appearance of IL-1, -3, -4 and TNF-α induced by intra-tracheal instillation of lipopolysaccharide and specific antigen in aqueous lung extracts [8, 10]. More recently, the authors of the present study have reported that RXM suppresses the ability of human peripheral blood T-cells to produce IL-4 and -5 (but not interferon-γ and IL-2) after costimulatory molecule stimulation [11]. These reports strongly suggest that macrolide antibiotics may exert anti-inflammatory effects and result in favourable modification of the clinical status of chronic inflammatory diseases.

It has been previously reported that an accumulation of both neutrophils and macrophages in the airways is an important feature of chronic inflammatory diseases, the levels of which are normalised after a period of successful macrolide therapy, along with the favourable modification of clinical status in these diseases [2, 3]. The recruitment of circulating polymorphonuclear leukocytes (*e.g.* neutrophils and macrophages) into inflammatory sites involves traversing both the capillary walls and the interstitium [12, 13]. To traverse these barriers, inflammatory cells adhere to endothelial cells and degrade extracellular matrix (ECM) proteins [12, 13]. The degradation of ECM proteins, including basement membrane proteins, is regulated by matrix metalloproteinases (MMP) and their inhibitors (tissue inhibitors of metalloproteinases (TIMP)), which are secreted by a wide variety of cells, including

inflammatory cells, epithelial cells and fibroblasts, in response to inflammatory stimuli [13, 14]. However, currently, the influence of macrolide antibiotics on MMP and TIMP production is not fully understood [15]. Therefore, the present study was undertaken to answer the unresolved questions regarding the favourable effects of macrolide antibiotics on chronic airway inflammatory diseases by examining the influence of RXM on MMP and TIMP production from fibroblasts in response to inflammatory stimuli *in vitro*.

Materials and methods

Macrolides

RXM was kindly donated (Aventis Pharmaceuticals Co., Ltd, Tokyo, Japan) as a preservative-free pure powder. RXM was dissolved in 100% ethyl alcohol at 20 mg·mL^{-1}, diluted with RPMI-1640 medium (Sigma Chemical Co., Ltd, St Louis, MO, USA), supplemented with 10% foetal calf serum (RPMI-FCS; Irvine Scientific Co., Ltd, Santa Ana, CA, USA) at 1 mg·mL^{-1} and stored at 4°C until use. All dilutions were prepared from this stock solution by diluting with RPMI-FCS just before use. Josamycin (JM) (Sigma Chemical Co., Ltd) was dissolved in RPMI-FCS in a similar manner.

Establishment of nasal polyp fibroblasts *in vitro*

Nasal polyp fibroblasts (NPF) were established according to a previously described method [16]. In brief, nasal polyp specimens were surgically obtained from seven male patients (mean±SD: 37±17 yrs) with chronic sinusitis, with written informed consent according to the protocol approved by the Ethics Committee of Showa University (Tokyo, Japan). Specimens (∼1 mm^2) were washed several times with PBS, supplemented with 200 U·mL^{-1} penicillin, 200 µg·mL^{-1} streptomycin and 5 µg·mL^{-1} amphotericin B. Samples were then plated at a density of 10 pieces in 100-mm tissue culture dishes that contained antibiotic-free RPMI-FCS. When a monolayer of fibroblast-like cells was found to be confluent, cells were trypsinised and re-plated into culture dishes at a concentration of 5×10^5 cells·mL^{-1} in a final volume of 10 mL. After confluency, the cells were diluted twice and passaged. The cells were characterised [16] and used as NPF.

Cell culture

Cells (passaged six to nine times) were washed several times with RPMI-FCS and introduced into each well of 24-well culture plates in triplicate, at a concentration of 2.5×10^5 cells·mL^{-1} in a volume of 1 mL and allowed to adhere for 24 h. The wells were washed twice with RPMI-FCS to remove dead and unattached cells. Fresh medium, which contained TNF-α and various concentrations of macrolide antibiotics, was then added into each well in a total volume of 2 mL. The plates were maintained at 37°C in a humidified atmosphere with 5% CO$_2$. The culture supernatants were removed after 24 h and stored at -40°C until use. Cells were cultured in a similar manner for examining nuclear factor (NF)-κB and activator protein (AP)-1 activities and mRNA expression, and were collected 4 and 12 h after culture, respectively. The cells were then stored at -80°C until use [17].

Assay for MMP and TIMP

MMP-2, -9 and TIMP-2 levels in culture supernatants were examined in duplicate using commercially available ELISA test kits (Amersham Biosciences Corp., Piscataway, NJ, USA), according to the manufacturer′s instructions. The sensitivity of each ELISA kit for MMP-2, -9 and TIMP-2 was 0.6 ng·mL^{-1}, 0.6 ng·mL^{-1} and 3.0 ng·mL^{-1}, respectively.

Assay for mRNA expression

Expression of mRNA was examined using RT-PCR. Poly A$^+$ mRNA was extracted from NPF with µMACS mRNA isolation kits (Milteny Biotec, GmbH, Bergisch Gladbach, Germany), according to the manufacturer′s instructions. The first-strand cDNA synthesis from 1 µg mRNA was performed with a Superscript cDNA synthesis kit (Invitrogen Corp., Carlsbad, CA, USA). Amplification of cDNA (1 µL) was performed with a Takara PCR Amplification kit (Takara Shuzo, Co., Ltd, Shiga, Japan) using specific primers for MMP-2, -9, TIMP-2 and β-actin in a final volume of 30 µL. The primers used for RT-PCR were 5′-AGATCTT-CTTCTTCAAGGACCGGTT-3′ (sense) and 5′-GGCTGGT-CAGTGGCTTGGGGTA-3′ (anti-sense) for MMP-2, 5′-CCCACATTTGACGTCCAGAGAAGAA-3′ (sense) and 5′-GTTTTTGATGCTATTGGCTGAGATCCA-3′ (anti-sense) for MMP-9, 5′-CTCGCTGGACGTTGGAGGAAAGAA-3′ (sense) and 5′-AGCCCATCTGGTACCTGTGGTTCA-3′ (anti-sense) for TIMP-2, and 5′-CGGAACCGCTCAT-TGCC-3′ and 5′-ACCCACACTGTGCCCATCTA-3′ for β-actin [18]. The PCR conditions were as follows: 4 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 58°C, and 30 s at 72°C. After PCR cycling, there was a DNA extension period of 4 min at 72°C [18]. Each PCR product (10 µL) was run on 3% agarose gels, visualised by a UV illuminator after SYBR Green staining (BioWhittaker Molecular Applications, Rockland, ME, USA) and photographed. The intensity of mRNA levels was corrected using β-actin transcripts, calculated by a densitometer.

Assay for NF-κB and AP-1 activities

NF-κB activity was analysed using a commercially available ELISA test kit (Active Motif, Co., Ltd, Carlsbad, CA, USA), which contains sufficient reagents and monoclonal antibodies against p50 and p65 subunits, according to the manufacturer′s recommended procedure. Briefly, nuclear extract (5 µg protein) from NPF was introduced into each well of 96-well microtitre plates precoated with oligonucleotide containing the NF-κB consensus site (5′-GGGAC-TTTCC-3′) in a volume of 20 µL, and incubated for 1 h at 25°C. After washing three times, 100 µL of monoclonal antibody against p50 or p65 was added to the appropriate wells, and incubated for a further 1 h at 25°C. Anti-IgG HRP-conjugate in a volume of 100 µL was then added and incubated for 1 h at 25°C. Absorbance at 450 nm was measured after the addition of tetramethylbenzine solution. AP-1 activity was also examined using a commercially available ELISA test kit (Active Motif, Co., Ltd) which contains sufficient reagents and monoclonal antibodies against Fra 1 and Jun B, according to the manufacturer′s instructions. Using the manufacturer′s data sheets, the amount of NF-κB and AP-1, bound to DNA can be measured by these two ELISA systems.

Statistical analysis

The difference between control and experimental data was analysed statistically using ANOVA, followed by Fisher′s PLSD test. A p-value <0.05 was considered significant.

Results

Production of MMP from NPF in response to TNF-α stimulation in vitro

The dose-response relationship of TNF-α stimulation on the ability of NPF to produce MMP in vitro was examined. NPF from seven donors were stimulated with various concentrations of TNF-α for 24 h and MMP levels in culture supernatants were examined by ELISA. As shown in figure 1a, addition of TNF-α at >10 ng·mL^{-1} into cell cultures enhanced the ability of NPF to produce MMP-2: culture supernatants from cells stimulated with TNF-α contained much higher levels of MMP-2 as compared with nonstimulated controls. In the case of MMP-9 production, addition of TNF-α <10 ng·mL^{-1} into cell cultures did not significantly affect MMP-9 production from NPF, however, TNF-α at 20 ng·mL^{-1} caused a significant increase in MMP-9 levels in culture supernatants (fig. 1b). The data in figure 1b also show that the optimum concentration of TNF-α needed to stimulate MMP-9 production was 20–35 ng·mL^{-1}.

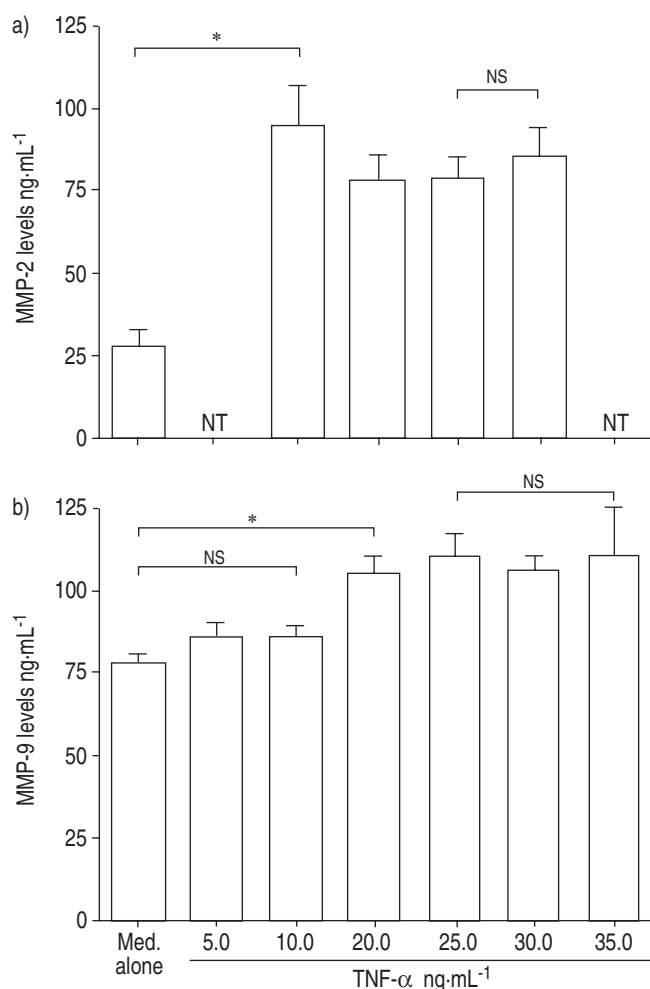### Influence of macrolide antibiotics on MMP and TIMP-2 production from NPF after TNF-α stimulation

The influence of RXM and JM on TNF-α-induced MMP-2 and -9 production from NPF was examined. NPF from seven donors were stimulated with 25 ng·mL^{-1} of TNF-α in the presence of various concentrations of either RXM or JM. MMP-2 and -9 levels in culture supernatants were examined 24 h later. As shown in figure 2a, RXM suppressed MMP-2 production from NPF, which is enhanced by TNF-α stimulation. The minimum suppressive concentration of RXM was 7.5 µg·mL^{-1}. JM could not suppress MMP-2 production even when 10 µg·mL^{-1} of the agent was added to cell cultures (fig. 2b). Addition of RXM into cell cultures at >5.0 µg·mL^{-1} completely suppressed MMP-9 production from TNF-α-stimulated NPF: MMP-9 levels in cell culture supernatants with >5.0 µg·mL^{-1} RXM were not significantly different to control cultures (fig. 3a). In contrast, JM did not suppress TNF-α-induced MMP-9 production from NPF, even when 10 µg·mL^{-1} was added to cell cultures (fig. 3b). The influence of macrolide antibiotics on TIMP production from NPF was

Fig. 1. – Dose response profile of tumour necrosis factor (TNF)-α on matrix metalloproteinase (MMP) production from nasal polyp fibroblasts (NPF) in vitro. NPF were stimulated with various concentrations of TNF-α for 24 h. MMP-2 (a) and -9 (b) concentrations in culture supernatants were assayed by ELISA. Data are presented as the mean±SE of seven different subjects. NT: not tested. NS: nonsignificant; *: p<0.05.
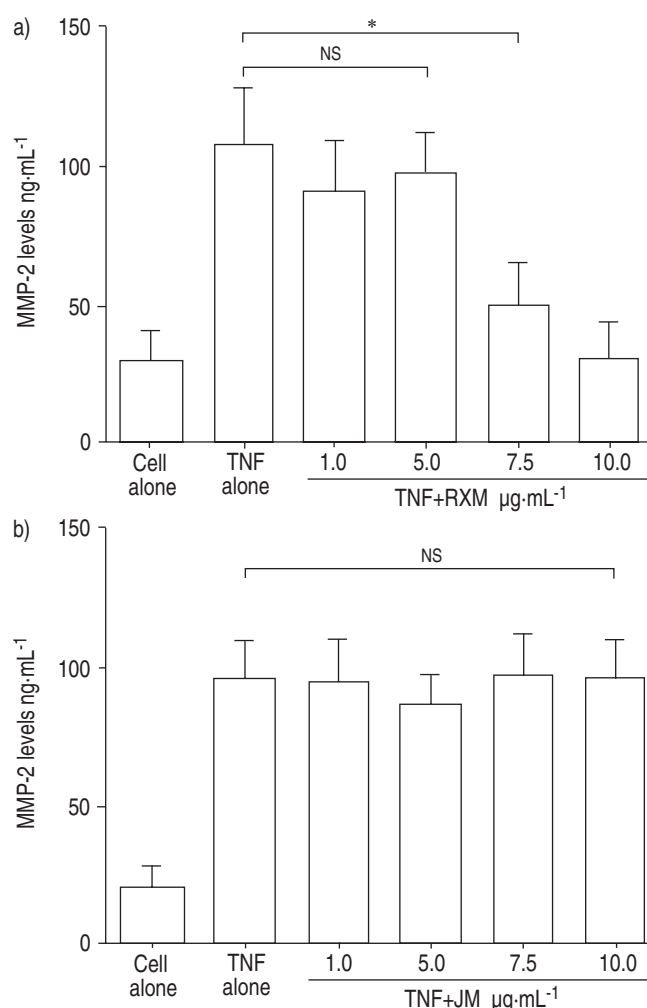
Fig. 2. – Influence of macrolide antibiotics on matrix metalloproteinase (MMP)-2 production from nasal polyp fibroblasts (NPF) in vitro. NPF were stimulated with tumour necrosis factor (TNF)-α in the presence of various concentrations of either roxithromycin (RXM; a) or josamycin (JM; b) for 24 h. MMP-2 levels in the culture supernatants were assayed by ELISA. Data are presented as the mean±SE of seven different subjects. NS: nonsignificant; *: p<0.05.
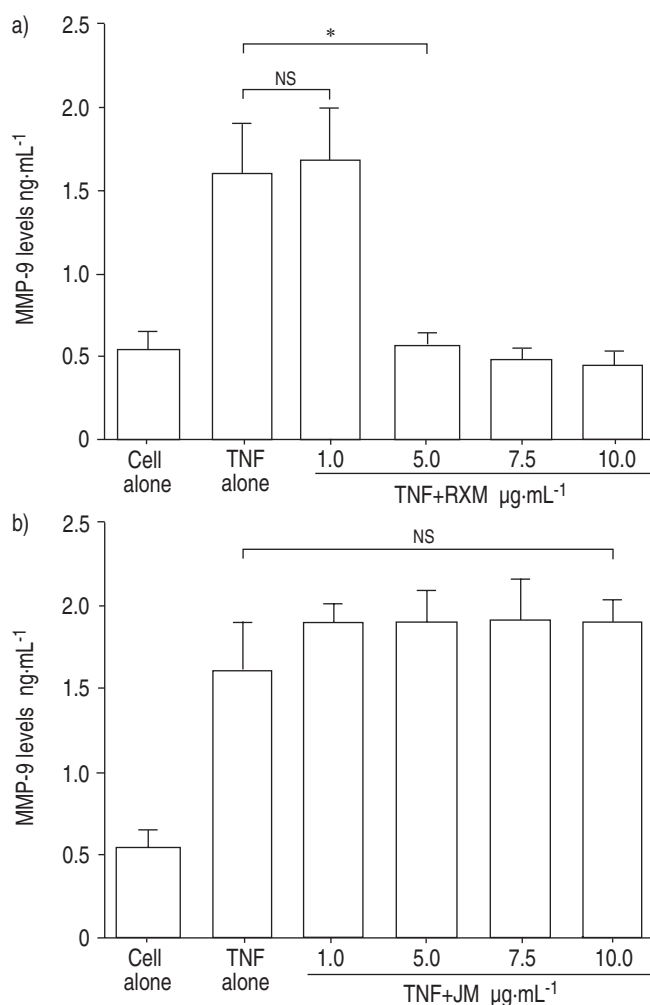
Fig. 3. – Influence of macrolide antibiotics on matrix metallo-proteinase (MMP)-9 production from nasal polyp fibroblasts (NPF) *in vitro*. NPF were stimulated with tumour necrosis factor (TNF)-α in the presence of various concentrations of either roxithromycin (RXM; a) or josamycin (JM; b) for 24 h. MMP-9 levels in the culture supernatants were assayed by ELISA. Data are presented as the mean±SE of seven different subjects. NS: nonsignificant; *: p<0.05.
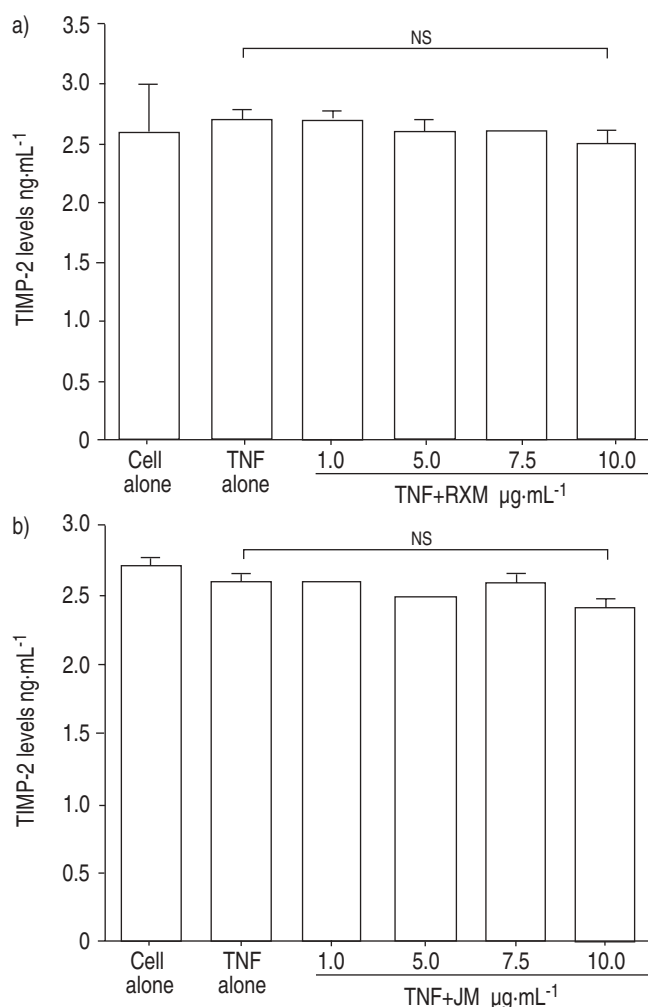

Fig. 4. – Influence of macrolide antibiotics on tissue inhibitors of matrix metalloproteinases (TIMP)-2 production from nasal polyp fibroblasts (NPF) *in vitro*. NPF were stimulated with tumour necrosis factor (TNF)-α in the presence of various concentrations of either roxithromycin (RXM; a) or josamycin (JM; b) for 24 h. TIMP-2 levels in the culture supernatants were assayed by ELISA. Data are presented as the mean±SE of seven different subjects. NS: nonsignificant.

also examined. The data in figures 4a and 4b show that the addition of macrolide antibiotics (RXM and JM) into cell cultures did not suppress TIMP production from NPF in response to TNF-α stimulation.

Suppressive activity of macrolide antibiotics on MMP and TIMP mRNA expression

This experiment was carried out to examine whether RXM suppressed mRNA expression and resulted in the inhibition of protein production, or whether RXM directly inhibited protein production. As shown in figure 5, RXM did not affect TNF-α-induced TIMP-2 mRNA expression even when 7.5 μg·mL^{-1} of the agent was added to cell cultures. However, MMP-2 mRNA expression was suppressed by RXM when the agent was added to cell cultures at 7.5 μg·mL^{-1}. This suppressive activity of RXM on mRNA expression was also observed for MMP-9 mRNA expression, in which the suppressive activity appeared at 5 μg·mL^{-1} (fig. 5). Graphs

showing the ratio of the target protein to β-actin obtained from seven different subjects confirmed these effects.

Suppressive activity of RXM on NF-κB and AP-1 activation

The influence of RXM on NF-κB and AP-1 activation in NPF by TNF-α stimulation *in vitro* was examined. NPF were stimulated with TNF-α in the presence of various concentrations of RXM for 4 h. The nuclear extracts were prepared, and NF-κB and AP-1 activities were examined. Addition of RXM at low concentrations (1 and 5 μg·mL^{-1}) into cell cultures did not affect NF-κB activation: optical density at 450 nm in experimental groups was similar to those in appropriate controls (fig. 6). However, higher concentrations (7.5 μg·mL^{-1}) of RXM caused significant inhibition of p50 and p65 activation. This inhibition was further increased when 10 μg·mL^{-1} RXM was added to cell cultures (fig. 6). RXM also exerted a dose-dependent suppressive effect on TNF-α stimulated AP-1 (Fra 1 and Jun B) activation (fig. 7).
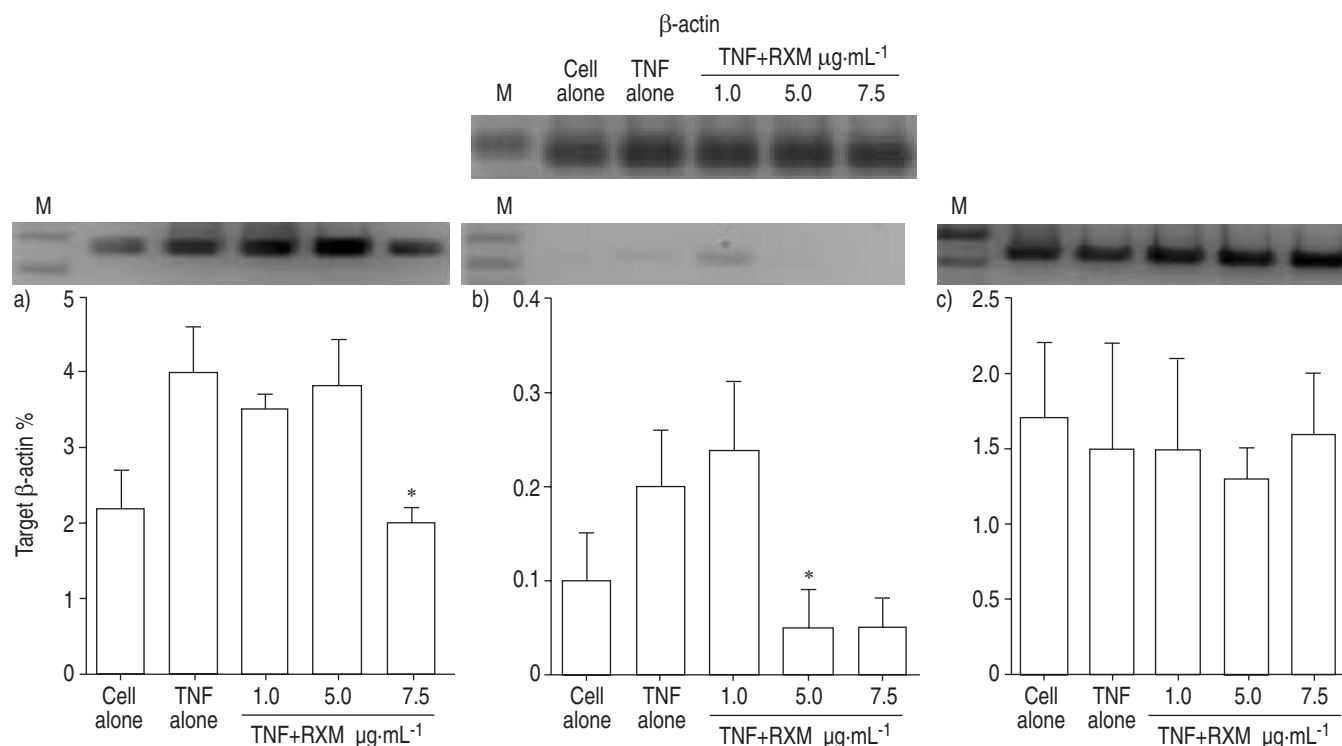
Fig. 5. – Influence of roxithromycin (RXM) on the expression of mRNA for matrix metalloproteinase (MMP)-2 (a), -9 (b), and tissue inhibitors of matrix metalloproteinases (TIMP)-2 (c) in nasal polyp fibroblasts (NPF) *in vitro*. NPF were stimulated with tumour necrosis factor (TNF)-α in the presence of various concentrations of RXM for 12 h. One typical result out of seven subjects is shown in the photographs. Intensity of mRNA levels corrected by β-actin is presented as the mean±SE of seven different subjects. M: marker. *: $p < 0.05$ as compared with TNF alone.

The minimum concentration of RXM which caused significant suppression of AP-1 activation was 5 µg·mL^{-1} (fig. 7).

Discussion

The present results clearly show that RXM suppresses MMP-2 and -9 production from NPF after TNF-α stimulation *in vitro*. However, this effect was not seen with JM. In addition, it has been shown that the suppressive activity of RXM is due to inhibition of NF-κB- and AP-1-dependent MMP mRNA expression, rather than direct inhibition of protein production.

Pharmacological studies have revealed that, after oral administration of RXM at doses of either 150 or 300 mg, plasma concentrations of this agent gradually increase and attain a plateau at 6.8 µg·mL^{-1} or 10.0 µg·mL^{-1}, respectively [19, 20]. These reports suggest that the findings of the present *in vitro* study may reflect the biological function of RXM *in vivo*.

Histological observations of the lungs from CF and DPB have revealed thickening of walls (including the basement membrane) of the respiratory bronchioles with predominant infiltration of polymorphonuclear leukocytes, especially neutrophils [3, 21]. These inflammatory changes were accompanied by oedema and extended to the peribronchiolar tissues [3, 21]. It is well accepted that chronic sinusitis is a chronic inflammatory disease of the upper airways, featuring inflammatory cell infiltration, modifications of epithelial differentiation and tissue remodelling, which includes basement membrane thickening, ECM accumulation and oedema [22, 23]. These morphological changes are linked to alterations in the activities of both MMP and their counter-regulatory inhibitors, TIMP. The MMP are a group of

zinc-dependent endopeptidases, which are capable of digesting the ECM and basement membrane [12–14].

Among the MMP family, MMP-2 and -9 more specifically degrade native type IV and V collagen and denatured collagens, as well as elastin, which are the most important components of the ECM and basement membrane in airway walls [12–14]. These MMP are produced by numerous cell types, including fibroblasts and neutrophils, in response to inflammatory stimuli, and mediate transmigration of inflammatory cells through the basement membrane to propagate inflammation [13, 24]. MMP also appear to be responsible for microvascular permeability leading to oedema and enhancement of cell transmigration [12, 24]. Recent *in vitro* studies clearly demonstrated that erythromycin could inhibit MMP-9 production from the human monocyte-like cell line U937 and mouse splenic macrophages, through the suppression of MMP-9 mRNA expression [15]. This suppressive activity of erythromycin on MMP-9 is also reported to correlate well with the inhibitory action of this agent on macrophage migration [15]. Therefore, it is reasonable to speculate that the inhibitory action of RXM on both MMP-2 and -9 production from NPF may be one of the therapeutic mechanisms by which macrolide antibiotics exert their effects in chronic inflammatory airway diseases. This suggestion may be supported by the finding that JM, which has been ineffective in the empirical treatment of DPB [2, 3], does not exert suppressive effects on MMP production. Most MMP are secreted from cells as inactive proenzymes, which are cleaved extracellularly to produce their active forms [25]. The extracellular activity of MMP is regulated by TIMP, which are secreted by the same cell types that produce MMP [25]. The present results clearly show that RXM cannot suppress the ability of NPF to produce TIMP-2, suggesting that MMP secreted in low amounts during macrolide treatment are
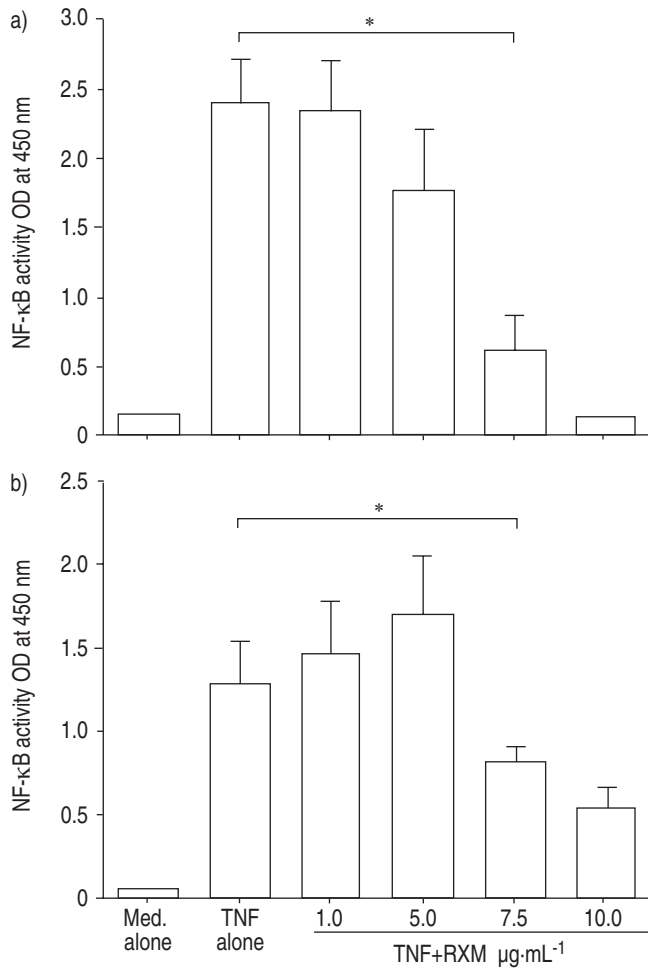
Fig. 6. – Influence of roxithromycin (RXM) on nuclear factor (NF)-κB activation in nasal polyp fibroblasts (NPF) *in vitro*. NPF were stimulated with tumour necrosis factor (TNF)-α in the presence of various concentrations of RXM for 4 h. p50 (a) and p65 (b) activities were examined by ELISA. Data are presented as the mean optical density (OD) at 450 nm±SE of seven different subjects. *: p<0.05.
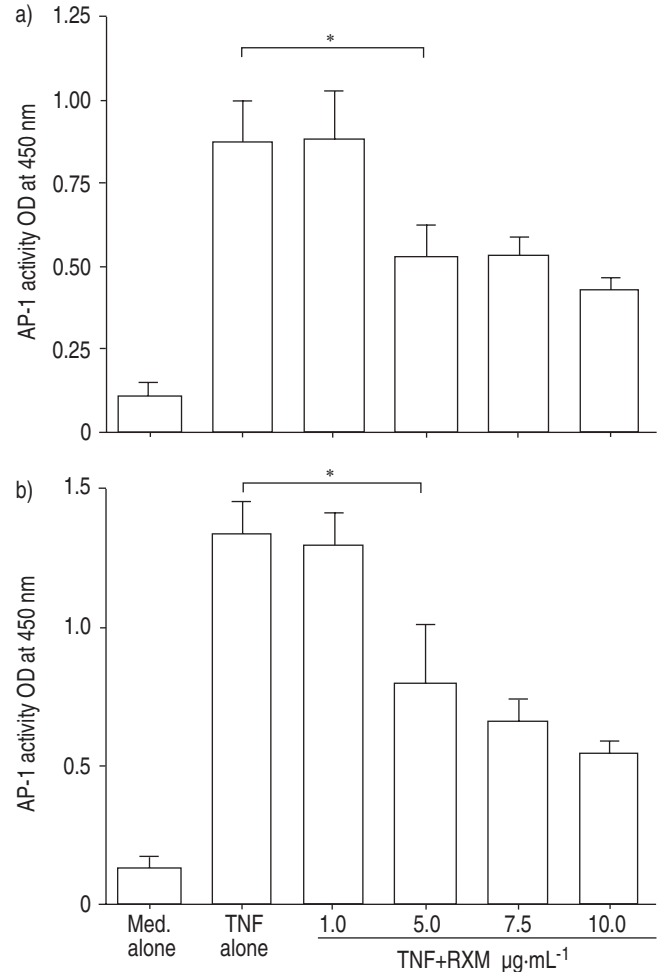

Fig. 7. – Influence of roxithromycin (RXM) on activator protein (AP)-1 activation in nasal polyp fibroblasts (NPF) *in vitro*. NPF were stimulated with tumour necrosis factor (TNF)-α in the presence of various concentrations of RXM for 24 h. Fra 1 (a) and Jun B (b) activities were examined by ELISA. Data are presented as the mean optical density (OD) at 450 nm±SE of seven different subjects. *: p<0.05.

inactivated by TIMP, and result in the inhibition of ECM remodelling and inflammatory responses.

MMP-2 and -9 share many common features, including identical substrate specificity, but their gene expression is differentially regulated due to the distinct structure of elements and promoters in their genes [26, 27]. MMP-2 gene expression requires translocation of the transcription factor NF-κB (but not AP-1) to the nucleus and binding to promoter regions, which causes the induction of mRNA expression [28, 29]. In contrast, AP-1 protein is essential for MMP-9 gene expression [28, 30]. It has been reported that erythromycin can inhibit the activation of transcription factors, NF-κB and AP-1 [17], suggesting that RXM inhibits the activation of NF-κB and AP-1 induced by TNF-α stimulation and results in suppression of MMP production. This suggestion may be supported, in part, by the present results, showing the suppressive activity of RXM (at concentrations equivalent to blood concentrations in the therapeutic range) on NF-κB (p50 and p65) and AP-1 (Fra 1 and Jun B) activation induced by TNF-α stimulation. It was recently reported that expression of α5β1 integrin, the receptor for fibronectin, one of the important basement components, enhances MMP gene expression in fibroblasts and macrophages [31, 32]. α5β1 integrin expression is also reported to be

mediated by NF-κB [32, 33], suggesting the possibility that RXM might diminish the expression of α5β1 integrin on the fibroblast cell surface and result in the inhibition of MMP production.

TNF-α is a multifunctional cytokine that plays a role in inflammation, immunity and a variety of diseases. It is also accepted that TNF-α activates several components implicated in cellular signal transduction. Binding of TNF-α to the type-1 TNF receptor causes an increase in intracellular Ca^{2+} concentrations through calcium influx [34], and results in activation of both NF-κB and AP-1, which are essential transcriptional factors for MMP production [28–30, 35]. Erythromycin is reported to inhibit an increase in Ca^{2+} concentrations in epithelial cells through the suppression of Ca^{2+} influx from the extracellular space [36]. Therefore, it is reasonable to speculate that RXM may suppress Ca^{2+} influx into NPF after TNF-α stimulation, resulting in the inhibition of the transcriptional factor (NF-κB and AP-1) activation responsible for MMP mRNA expression.

In the cytosol, NF-κB is found in an inactive form bound to an inhibitory molecule, IκB [37]. Stimulation of cells with TNF-α causes dissociation of NF-κB from IκB through the activation of mitogen-activated protein kinase [38]. After

translocation into the nucleus, NF-κB is reduced by a cellular reducing catalyst, thioredoxin, through redox control mechanisms [37, 38]. The reduced form of NF-κB then binds to the promoter region of the NF-κB-dependent gene [37, 38]. Recently, it has been reported that erythromycin cannot interfere with the dissociation of NF-κB from IκB induced by TNF-α stimulation in human bronchial epithelial cells [39]. Erythromycin has also been reported to be unable to suppress the translocation of NF-κB to the nucleus, whereas erythromycin significantly suppresses the DNA-binding activity of reduced NF-κB and results in the inhibition of mRNA expression in an NF-κB-dependent manner [39]. From these observations, it may be interpreted from the present results that RXM prevents the binding of NF-κB to promoter regions in DNA and results in inhibition of MMP mRNA expression.

Recent immunohistological observations have revealed that stromal cells of squamous cell carcinoma of the lung are highly positive for MMP-1 and -9, and that fibroblasts located in the tumour-surrounding tissues are also positive for MMP-2 [40], suggesting the involvement of these MMP in highly invasive and potentially metastatic squamous cell carcinomas of the lung. Taken together, the present results may suggest that long-term use of the 14-membered macrolide antibiotics, erythromycin and RXM, in patients with squamous cell carcinoma of the lung prevent tumour cell growth, invasion and metastasis. This suggestion may be supported by the findings that rapamycin, a 31-membered macrolide antibiotic with immunosuppressive activities, can inhibit metastatic tumour cell growth and can control the growth of established tumours, when the agent was injected into mice for 7 days [41].

In conclusion, the results from the present study demonstrate that the therapeutic mode of action of macrolide antibiotics on chronic inflammatory airway diseases is, in part, due to its suppressive activity on matrix metalloproteinase-2 and -9 production. Thus, macrolide antibiotics may reduce the extracellular spread of inflammation through the inhibition of matrix metalloproteinases.

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