The Effect of Different Dosage Regimens of Roxithromycin on its Anti-Inflammatory Activity and Possible Mechanisms of Action in Acute Pleurisy and Chronic Arthritis in Rats

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Abstract

Many of the steps in the inflammatory cascade are reported to be controlled by soluble regulatory molecules (serotonin, histamine and leukotriene, etc.) known as inflammatory or chemical mediators. Furthermore, much evidence clearly shows that T cells play a pivotal role in initiation, driving and maintenance of all these processes by elaboration of several types of cytokines. Several studies have shown that long-term administration of macrolide antibiotics can favorably modify the clinical condition of inflammatory diseases. Although the precise therapeutic mechanisms of macrolides are not well understood, Roxithromycin, a macrolide with better plasma concentrations and higher tissue concentrations was tested in an acute and chronic rat animal models of inflammation.

Aim: The aim of the present study was to elucidate the effect of different dosage regimens of roxithromycin on its anti-inflammatory activity and possible mechanism of action in experimental models of acute pleurisy and chronic arthritis in rats.

Methods: Male albino rats were subjected to two different models of inflammation. Acute pleurisy was induced by injecting carrageenan into the pleural cavity of the rat. One hour before carrageenan injection and 24 and 48h thereafter, rats were given roxithromycin in single oral daily ascending dosage schedule of 2.5, 5, 10, 20 and 40mg/kg. Control group received only distilled water. The animals were sacrificed 72 hours after carrageenan and the pleural fluid was aspirated, its volume, total and differential cell count and TNF-α levels were measured.

Chronic soft tissue inflammation and arthritis were induced by injecting formalin into the subplantar region of the left hind paw of the rat on day 1 and day 3 of the experiment. Rats were treated with roxithromycin in the same previous single oral daily doses for the whole length of the experiment 21 days. The increase in the paw volume of each treated group was measured using Digital Plethysmometer. Blood samples were collected for measurement of serum TNF-α level. One day before formalin injection and on the 21st day of formalin injection, the left hind paw was imaged antero-posteriorly by X-ray. On the 21st day the animals were sacrificed and their left hind paws were excised for histopathological examination.

Results: Administration of roxithromycin in a dose of 2.5mg/kg/day resulted in insignificant changes neither in acute nor chronic inflammation; while 5, 10, 20 and 40mg/kg/day significantly reduced the pleural exudate volume, the total leukocyte number and the TNF-α level in the pleural exudates. Also roxithromycin in previous doses attenuated the increase in paw volume, serum TNF-α level and the signs of inflammation; soft tissue edema, joint deformity and destruction were correlated dose dependently with maximum response observed on day 21.

Conclusion: Roxithromycin has anti-inflammatory activity in the models of acute carrageenan pleurisy and chronic formalin-induced arthritis dose dependently with maximum response on day 21. Macrolides affect several pathways of the inflammatory process, including the prostaglandins formation, the migration of neutrophils and the production of proinflammatory cytokine TNF-α.

Key Words: Macrolides – Roxithromycin – Inflammation – Models of inflammation in rats.

Introduction

THE term macrolides encompasses a diverse family of unrelated compounds with large macrolactam rings. This very large class (>2000 compounds) comprises both natural substances isolated from fungi and other organisms, as well as synthetic molecules of a similar structure [1].

Of this group of drugs, the macrolide antibiotics; referred to as macrolides are the most commonly clinically used agents. These include erythromycin, roxithromycin and clarithromycin as typical members of the 14-member class, and azithromycin as the prototypical 15-member compound. Macrolide antibiotics bind to 50S ribosomal subunit of both prokaryotes and eukaryotes, inhibiting transpeptidation or translocation of nascent peptides [2].

Apart from their antibacterial activity, macrolides exhibit a broad spectrum of pharmacological
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Effects including anti-inflammatory and immunomodulatory activities [3,4]. Macrolide antimicrobial agents have the potential to serve a unique role in the management of chronic inflammatory lung disorders including diffuse panbronchiolitis, cystic fibrosis, bronchial asthma and chronic obstructive pulmonary disease [8]. They have been used to treat chronic rhinosinusitis with or without lower respiratory tract disease and it is thought that this is primarily due to their ability to modulate chronic inflammation [6]. Also, macrolides are effective in treating rosacea, an inflammatory skin disease [7]. In several studies patients with unstable angina treated with roxithromycin showed a significant reduction in major ischemic events possibly due to attenuation of the persistent inflammation in the atherosclerotic plaque [8].

Recent reports demonstrated that roxithromycin (RXM), a semi-synthetic macrolide antibiotic, may exert immunomodulatory effects that differ from classic immunosuppression through inhibiting the production of proinflammatory mediators independent of its antibacterial activity. However, the mechanisms and optimal dosage regimens remain unclear [9].

Aim of the work:

The present work was designed to study the possible anti-inflammatory mechanisms of different dose regimens of roxithromycin in experimental models of acute pleurisy and chronic arthritis in rats.

Material and Methods

This study was approved by our institution’s (Kasr El-Eini Hospital) Animal Care Committee and the guidelines were strictly adhered to.

Drugs and reagents:

- Roxithromycin (Sigma Aldrich, Germany): The pure white roxithromycin powder was freshly prepared before administration by dissolving in distilled water.
- Carrageenan (sigma Aldrich, Germany): The pure \(\lambda\)-carrageenan powder was freshly prepared before administration by dissolving it in sterile saline solution.
- Ether (S D line-chem limited, India).
- Formalin 37-40% (Abou Za'abale Company for Industrial Detergents, Egypt).
- Heparin (Amoun Pharmaceutical, Egypt).
- Kits: Rat Tumor Necrosis Factor (TNF-\(\alpha\)) ELISA kit (Ray Biotech Inc., USA).
- Apparatus: Digital Plethysmometer (Panlab, Spain).

Animals:

Male albino rats, weighing 200-220g, were used. They were housed in cages at ordinary room temperature; exposed to natural daily light/dark cycle, fed standard laboratory chow and tab water ad libitum. They were acclimatized for 1 week and randomly allocated into groups. Each group consisted of 6 rats.

Model of acute inflammation: Carageenan induced pleurisy in rats [10]:

Under ether anesthesia a small incision under the right arm between the seventh and eighth rib was done. The wound was opened and a further shallow incision was made into the exposed intercostals muscle. 0.1ml of 2% carageenan solution was injected into the pleural cavity through this incision. One hour before carageenan injection and 24 and 48 hours thereafter, rats were treated with single dose of roxithromycin p.o. via gastric gavage; control group received an equal volume of the vehicle. The animals were sacrificed 72 hours after carageenan. Under ether anesthesia an incision in the skin over the xiphisternal cartilage was made. The cartilage was lifted with a forceps and a small cut is made with scissors under it to gain access into the pleural cavity. 0.1ml of heparin was injected into the pleural cavity through this cut. The fluid was aspirated out of the cavity using a pipette. Exudates with blood contamination were rejected. The exudates volumes were measured, the samples were centrifuged at 800g for 10min, and the cell pellet was resuspended in saline for total and differential cell count. The supernatants were then aliquoted and stored at \(-80^\circ\)C until use for determination of TNF-\(\alpha\) level.

Animals were divided into 6 groups:

- Group (1): Rats were given 1ml of distilled water orally 1 hour before carageenan injection and 24 and 48 hours thereafter.

In Groups (2), (3), (4), (5) and (6): Rats were given RXM in the doses of 2.5, 5, 10, 20 and 40 mg/kg [equivalent to doses 150, 300, 600, 1200 and 2400mg in man [11,12] dissolved in 1ml of distilled water as single daily dose p.o. via gastric gavage 1 hour before carageenan injection and 24 and 48 hours thereafter.

Assessment of the following parameters was conducted 72 hours after carageenan:

- The volume of the pleural exudate (0.1ml heparin subtracted).
WBC count in the pleural exudate, using a Flow cytometer.

TNF-α level in the pleural exudate with an ELISA kit according to the manufacturer’s instructions [13].

Model of chronic inflammation: Formalin induced arthritis in rats [14]:

Chronic soft tissue inflammation and arthritis were induced by injecting 0.1ml of formalin 2% solution into the subplantar region of the left hind paw of the rat on day 1 and day 3 of the experiment.

Animals were divided into 7 groups:

Group (1): Normal rats were given RXM 10 mg/kg dissolved in 1ml of distilled water in a once per day oral dose the whole length of the experiment.

Group (2): Rats were given 1ml of distilled water orally once per day starting 1 hour before formalin injection and daily for the whole length of the experiment.

In groups (3), (4), (5), (6) and (7) rats were subjected to subplantar formalin injection and were given RXM 2.5, 5, 10, 20 and 40mg/kg equivalent to 150, 300, 600 and 1200 and 2400mg in man [11,12] dissolved in 1ml of distilled water orally in single daily doses starting 1 hour before formalin injection and daily for the whole length of the experiment [13].

The following parameters were assessed:

- The mean increase in the paw volume of each treated group was measured at days 1, 3, 7, 14 and 21 by using Digital Plethysmometer.
- Blood samples were collected for measurement of TNF-α level at days 1, 3, 7, 14 and 21.
- One day before formalin injection and on the 21st day of formalin injection, the left hind paw was imaged antero-posteriorly by X-ray.
- On the 21st day the animals were sacrificed and their left hind paws were excised for histopathological examination carried out after fixation of the tissue in 10% formalin, it was processed by using automatic histochinite and embedded in paraffin. Preparations of 5 micron sections were stained by hematoxylin and eosin. Histopathological microscopic examination was done and results recorded.

Statistical analysis:

Statistical analysis was performed with use of the SPSS 9.0. (SPSS, Chicago, IL. USA) software package. Group mean values were compared by one-way analysis of variance (ANOVA) followed by Tukey’s Multiple Comparison Test. Data was expressed as mean ± standard deviation of the mean. A p-value of less than 0.05 was considered as significant.

Results

Carrageenan induced pleurisy in rats:

The injection of 0.2ml of 5% λ-carrageenan into the pleural cavity of rats produced inflammatory reaction characterized by exudate formation and cell migration. In the control untreated group, the volume of the exudate collected after 72 hours was in the range of 0.8-1.3ml (mean ± SD, 1.12 ± 0.09ml). The total leukocyte number (90% neutrophils) that migrated into the pleural cavity was 110.83 ± 11.41 (x10⁶) cells/rat. The TNF-α level in the pleural exudate was 605.83 ± 17.44pg/ml (Table 1).

Administration of RXM in a dose of 2.5mg/kg/day produced insignificant pleural fluid parameter changes; while RXM in the doses of 5, 10, 20 and 40mg/kg/day orally for 3 days significantly reduced the pleural exudate volume, the total leukocyte number and the TNF-α level in the pleural exudate dose dependently but with insignificant difference between rat group that received 20 and rat group that received 40mg/kg/day (Table 1); for the doses 5, 10, 20 and 40mg/kg/day, the percentage decrease of the pleural exudate volume were 30.35, 41.96, 59.82 and 63.4% respectively, the total leukocyte numbers (90% neutrophils) were 13.53, 25.86, 36.39 and 38.2% respectively and the TNF-α levels in the pleural fluids were 28.20, 43.61, 51.72 and 53.7% respectively (Table 1, Fig. 1).

Formalin induced arthritis in rats:

Injection of formalin into the plantar surface of the hind paw of the rats on days 1 and 3 (group 2) produced marked increase in paw volume most marked on day 7 (Table 2) with marked increase in serum TNF-α level starting at day 3 (Table 3), marked paw tissue inflammation that involved all layers starting with skin covering, fibromuscular tissue and synovial membrane of the small joints of the paw (Photo 1). The histopathological study also showed severe inflammatory cell infiltration (leucocytes and lymphocytes mainly), congested blood vessels and soft tissue edema (Photo 4) with radiological manifestations of inflammation and arthritis in the form of marked soft tissue edema, periarticular bone resorption, narrowing of joint spaces and joint deformities (Photo 2).
Administration of RXM in various doses one hour before formalin injection and daily for the whole length of the experiment resulted in insignificant early effects (on days 1 and 3) between groups. In a dose of 2.5mg/kg/day, the treatment was ineffective; RXM produced insignificant decrease in paw volume, serum TNF-α level as well as same histopathological changes (Photo 4) compared to untreated. Roxithromycin at doses of 5, 10, 20 and 40mg/kg/day significantly attenuated the increase in paw volume (Table 2, Fig. 2), serum TNF-α level (Table 3, Fig. 3) but with delay in the effect and the signs of inflammation, soft tissue edema, joint deformity and destruction (Photos 2-7) dose dependently with maximum response observed on day 21. There was insignificant difference between doses 20 and 40mg/kg/day (Groups 6 and 7) (Table 3, Fig. 3).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pleural fluid volume (ml)</th>
<th>Pleural fluid TLC (x 10^6) cells/rat</th>
<th>Pleural fluid TNF-α (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range (min-max)</td>
<td>Mean ± SD</td>
<td>% reduction</td>
</tr>
<tr>
<td>Group 1 (Carrageenan pleurisy-no treatment)</td>
<td>0.8-1.3</td>
<td>1.12±0.09</td>
<td>110.83±11.41</td>
</tr>
<tr>
<td>Group 2 (Carrageenan pleurisy-RXM 2.5mg/kg/day)</td>
<td>0.8-1.3</td>
<td>1.10±0.17</td>
<td>1.8%</td>
</tr>
<tr>
<td>Group 3 (Carrageenan pleurisy-RXM 5mg/kg/day)</td>
<td>0.7-0.8</td>
<td>0.78±0.07*</td>
<td>30.35%</td>
</tr>
<tr>
<td>Group 4 (Carrageenan pleurisy-RXM 10mg/kg/day)</td>
<td>0.5-0.9</td>
<td>0.65±0.1*</td>
<td>41.96%</td>
</tr>
<tr>
<td>Group 5 (Carrageenan pleurisy-RXM 20mg/kg/day)</td>
<td>0.4-0.6</td>
<td>0.45±0.08**</td>
<td>59.82%</td>
</tr>
<tr>
<td>Group 6 (Carrageenan pleurisy-RXM 40mg/kg/day)</td>
<td>0.4-0.6</td>
<td>0.41±1.09**</td>
<td>63.4%</td>
</tr>
</tbody>
</table>

*p-value <0.05 in comparison to group 1 & 2. •p-value <0.05 in comparison to group 3 & 4. ‡p-value <0.05 in comparison to day 7&14.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean change in paw volume ± SD (mm) in relation to time of formalin injection</th>
<th>% reduction in relation to group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 3</td>
</tr>
<tr>
<td>Group 1 (Normal rats + RXM)</td>
<td>0.1±0.03</td>
<td>0.09±0.03</td>
</tr>
<tr>
<td>Group 2 (Formalin arthritis – no treatment)</td>
<td>0.83±0.02</td>
<td>1.88±0.03</td>
</tr>
<tr>
<td>Group 3 (Formalin arthritis – RXM 2.5mg/kg/day)</td>
<td>0.87±0.05</td>
<td>1.91±0.07</td>
</tr>
<tr>
<td>Group 4 (Formalin arthritis – RXM 5mg/kg/day)</td>
<td>0.82±0.07</td>
<td>1.83±0.05</td>
</tr>
<tr>
<td>Group 5 (Formalin arthritis – RXM 10mg/kg/day)</td>
<td>0.79±0.08</td>
<td>1.87±0.09</td>
</tr>
<tr>
<td>Group 6 (Formalin arthritis – RXM 20mg/kg/day)</td>
<td>0.81±0.07</td>
<td>1.76±0.05</td>
</tr>
<tr>
<td>Group 7 (Formalin arthritis – RXM 40mg/kg/day)</td>
<td>0.76±0.06</td>
<td>1.82±0.07</td>
</tr>
</tbody>
</table>

*p-value <0.05 in comparison to groups 2&3. •p-value <0.05 in comparison to groups 4&5. ‡p-value <0.05 in comparison to day 7&14.
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Table (3): Effect of Roxithromycin on rat serum TNF-α level [Mean change ± SD (pg/ml) and % reduction in relation to group 2] in formalin induced arthritis in rats (n=6).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean level of serum TNF-α ± SD (pg/ml) in relation to time of formalin injection</th>
<th>% reduction in relation to group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1 (Basal TNF-α level)</td>
<td>Day 3</td>
</tr>
<tr>
<td>Group 1 (RXM 1 0mg/kg/day – no formalin)</td>
<td>24.67±4.6</td>
<td>23±3.61#</td>
</tr>
<tr>
<td>Group 2 (Formalin arthritis – no treatment)</td>
<td>23.33±2.98</td>
<td>79.33±2.58</td>
</tr>
<tr>
<td>Group 3 (Formalin arthritis – RXM 2.5mg/kg/day)</td>
<td>24.17±1.11</td>
<td>72.83±4.42</td>
</tr>
<tr>
<td>Group 4 (Formalin arthritis – RXM 5mg/kg/day)</td>
<td>22.17±2.79</td>
<td>69.50±3.53*</td>
</tr>
<tr>
<td>Group 5 (Formalin arthritis – RXM 10mg/kg/day)</td>
<td>23±3.62</td>
<td>64.83±3.04*</td>
</tr>
<tr>
<td>Group 6 (Formalin arthritis – RXM 20mg/kg/day)</td>
<td>24.50±2.71</td>
<td>56±3.39*</td>
</tr>
<tr>
<td>Group 7 (Formalin arthritis – RXM 40mg/kg/day)</td>
<td>24.83±1.71</td>
<td>54±4.9*</td>
</tr>
</tbody>
</table>

# p-value <0.05 in comparison to all groups. † p-value <0.05 in comparison to group 4.
* p-value <0.05 in comparison to groups 2 & 3. ‡ p-value <0.05 in comparison to groups 4 & 5.

Fig. (1): Percentage reduction produced by roxithromycin on pleural fluid parameters: Volume, TLC and TNF-α levels compared to group 1 (carrageenan pleurisy with no treatment) (n=6).

• p-value <0.05 in comparison to group 3 & 4.
† p-value <0.05 in comparison to group 3.

Fig. (2): Percentage inhibition produced by roxithromycin on paw volume in formalin induced arthritis in rats compared to group 2 (Formalin arthritis with no treatment).

• p-value <0.05 in comparison to groups 4 & 5.
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Fig. (3): Percentage inhibition produced by roxithromycin on rat serum TNF-α level compared to group 2.

* p-value <0.05 in comparison to groups 4 & 5.
† p-value <0.05 in comparison to group 4.

Photo (1): Hind paw of the same rat: Normal right and left Formalin injected. Formalin produced marked rat paw tissue inflammation that involved all layers starting with skin covering, fibromuscular tissue and synovial membrane of the small joints of the paw.

Photo (2): X-ray (antero-posterior) of rat hind paw showing untreated formalin induced arthritis in the form of: Marked soft tissue oedema, periarticular bone resorption and lowered bone density, narrowing of joint spaces and joint deformities; and effect of roxithromycin 10mg/kg/day on formalin induced arthritis.

Photo (3): Histopathological changes of normal rat hind paw (H&E x 200).
Photo (4): Histopathological changes (H&E) of group 2 (Formalin arthritis – no treatment) and group 3 (roxithromycin 2.5mg/kg/day) rat hind paw. The histopathological photo shows severe inflammatory cell infiltration (leucocytes and lymphocytes mainly), congested blood vessels and soft tissue oedema.

Photo (5): Histopathological changes (H&E) of group 4 (roxithromycin 5mg/kg/day) rat hind paw. The histopathological photo shows moderate edema, congested blood vessels and infiltration of inflammatory cells.

Photo (6): Histopathological changes (H&E) of group 5 (roxithromycin 10mg/kg/day) rat hind paw. The histopathological photo shows minimal edema with inflammatory cells infiltration in soft tissues.

Photo (7): Histopathological changes (H&E) of group 6 (roxithromycin 20mg/kg/day) and group 7 (roxithromycin 40mg/kg/day) rat hind paw. The histopathological photo shows marked improvement in edema, inflammation and congested blood vessels.
Discussion

After a long history of use as antimicrobial medications, macrolide antibiotics have not yet given up all their secrets. Interest in their therapeutic potential in inflammatory diseases (including non-infectious diseases) has generated abundant fundamental research and therapeutic trials worldwide [15].

The present study evaluated the anti-inflammatory effects of different doses of RXM, a semi-synthetic 14-membered ring macrolide antibiotic, its mechanisms as anti-inflammatory and its ability to reduce the production of TNF-α; a pro-inflammatory cytokines in both acute and chronic inflammatory models.

The inhibition of carrageenan induced inflammation in rats is an established model for evaluating anti-inflammatory drugs, which act through an anti-edematous effect. The development of carrageenan induced inflammation is biphasic [16]; the first phase occurs within one hour of carrageenan inflammation and is attributed to the release of cytoplasmic enzymes, histamine and serotonin, from the mast cells. The second phase (>1.0h) is mediated by an increased release of prostaglandins in the inflammatory area and continuity between the two phases is provided by kinins.

Also the inhibition of edema induced by formalin in rats is one of the most suitable test procedures to screen anti-arthritic and anti-inflammatory agents, as it closely resembles human arthritis [17]. Arthritis induced by formalin is a model used for the evaluation of an agent with probable antiproliferative activity.

The present results revealed that prior administration of RXM in a dose of 2.5mg/kg/day produced insignificant changes in both acute and chronic inflammation.

Roxithromycin 5, 10, 20 and 40mg/kg/day for 3 days significantly reduced the pleural exudate volume, the total leukocyte number and the TNF-α in the pleural exudate dose dependently but with insignificant change between 20 and 40mg/kg/day; these changes were measured 72 hours after carrageenan induced pleurisy which probably suggest a possible inhibition of cyclooxygenase synthesis by RXM in the second phase of acute inflammation.

Roxithromycin 5, 10, 20 and 40mg/kg/day attenuated both the increase in paw volume and the increase in the serum TNF-α also dose dependently, with maximum response observed on day 21 in the chronic model of arthritis. Both the histopathological and the radiological studies revealed attenuation of the inflammation after the administration of RXM. The results of the formalin test suggested the effect of RXM on formalin induced cell damage and accordingly, arthritic conditions.

Ianaro et al. [13] studied the anti-inflammatory activity of four macrolide antibiotics, roxithromycin, clarithromycin, erythromycin, and azithromycin, and their ability to reduce the production of proinflammatory mediators and cytokines both in vivo and in vitro. Rat carrageenin pleurisy was used as a model of acute inflammation, and the inflammatory reaction was induced in either normal or adrenalectomized animals. The doses of roxithromycin (20 and 40mg/kg), clarithromycin (40mg/kg), and erythromycin (40mg/kg), which significantly reduced exudate volume and leukocyte accumulation in normal rats, exhibited an identical inhibitory effect in adrenalectomized rats, showing that the anti-inflammatory activity of macrolides was not dependent on the stimulation of endogenous corticoid production. Furthermore, in normal rats, the amounts of prostaglandin (PG) E2, NO (stable metabolites of nitric oxide NO in the pleural exudates), and TNF-α in pleural exudates were significantly reduced by these macrolides. They concluded that the inhibition of the prostanoid pathway may contribute, at least in part, to the anti-inflammatory effect of macrolides, although it has been shown that these agents suppress inflammation through mechanisms different from conventional nonsteroidal anti-inflammatory drugs.

Suzaki et al. [18] induced pneumonia by the inhalation of lipopolysaccharide (LPS) in mice after pre-treatment with RXM at 2.5mg/kg/day for 5-12 weeks. They determined the interleukin 1 β (IL-1β) and TNF-α levels in a lung extract and reported that the production of these cytokines was inhibited in mice receiving RXM for 7 weeks or more. Konno et al. [19] conducted the same experiment except that the dose was doubled to 5mg/kg/day, and they stated that the production of TNF-α was inhibited after 21 days of administration. These facts suggested that the in vivo inhibitory effects of RXM on IL-1β and TNF-α are affected by the period of administration as well as the dose. Takashi and his fellow workers [20] evaluated the inhibitory effect of RXM on the production of interleukin IL-1β, IL-6 and TNF-α in a murine tibial osteomyelitis model using Staphylococcus aureus. The levels of IL-1β, IL-6 and TNF-α in infected bone with and without roxithromycin were considerably greater than those in uninfected bone (control) with some variations in timing. The
roxithromycin administration group showed significantly lower IL-1β levels in bone than the non-administration group at 7 and 14 days. The TNF-α levels in the roxithromycin administration group did not differ from those in the non-administration group up to 14 days, but were significantly lower at 21 and 28 days. In contrast, the IL-6 levels in the roxithromycin administration and non-administration groups did not differ significantly at any point of measurement.

In the present study, the observation that there was no increase in response either in acute or chronic inflammation with the dose of 40mg/kg/day could be caused by a non linear absorption of RXM. With increasing doses in human in the range 150 to 300mg (equivalent to 2.5, 5mg/kg in rats), peak plasma levels and area under the curve (AUC) do not increase in proportion to the dose [21]. Many factors can cause interspecies differences in oral bioavailability including differences in the dissolution characteristics of the dosage form, the pH of the gastrointestinal contents, the stability of the active ingredient in the gastrointestinal tract and the extent of drug hydrolysis or metabolism prior to its reaching the systemic circulation [22].

In the model of chronic inflammation, the delay in the effect of RXM on the decrease in serum TNF-α level arises from the indirect response of the cytokine production by RXM. Indeed, this is the most reasonable explanation, because RXM acts by inhibition of cytokines mRNA transcription [18]. This finding was in agreement with Guchelaar et al. [23] report on the inhibitory effect of erythromycin on TNF-α and interleukin 6 (IL-6) productions in human.

The reduction in TNF-α in rat pleural exudate and serum is in agreement with previous reports showing that the systemic administration of macrolides in animals and humans down-regulates the production of proinflammatory cytokines, including TNF-α and interleukin 1 (IL-1) [19,26]. The suppressive effect of macrolides on the production of proinflammatory cytokines such as TNF-α, IL-6, and IL-1 has been extensively studied in vitro [25-27]. These reports stated that RXM inhibited the production of IL-1/β, IL-6 and TNF-α in a dose-dependent manner.

The administration of single p.o. doses of RXM in man at 150, 300 and 450mg (approximately 2.5, 5 and 7.5mg/kg), gave the mean Cmax values as 6.6±7.9, 9.1±10.8 and 12.2 µg/ml, respectively [11,12,28]. The minimum concentration of RXM exhibiting the inhibitory effects on the production of cytokines varies between reporters, from 0.05 to 3.1 µg/ml. Serum concentrations of RXM >0.5 µg/ml is the suggested cut-off point separating sensitive from resistant human pathogens [12,29].

Yasuyo et al. [30] evaluated the effect of RXM on T cell functions and the inflammatory responses in mice with collagen induced arthritis (CIA). RXM did not affect the production of Th1-type and Th2-type cytokines, whereas it specifically inhibited production of proinflammatory cytokines such as tumor necrosis factor-α and interleukin 6 (IL-6) by T cells and macrophages. RXM inhibited T cell migration. They found that RXM treatment of mice with CIA reduced the severity of arthritis and serum level of IL-6, as well as leukocyte migration into the affected joints and destruction of bones and cartilage.

The subcellular mechanism of the anti-inflammatory effect of macrolides remains unknown. The fact that there are several distinct inflammatory pathways, and that each of which proceeds via a cascade of biological events, makes it difficult to identify which of these events is the target of macrolides and the definite mechanism of their anti-inflammatory action.

Ou and coworkers [31] demonstrated that RXM inhibits the pulmonary inflammatory response and airway mucus hypersecretion induced by LPS. They speculated that the inhibitory effect of RXM on airway mucus hypersecretion may be mediated through reduction of nuclear Factor-κB (NF-κB) activation, neutrophil infiltration and release of inflammatory cytokines in the lung.

Since macrolide antibiotics exhibit their antimicrobial activity by interfering with the protein production of microorganisms, interference with protein production may be one mechanism by which cytokine production is influenced by macrolide antibiotics. Inhibition of cytokine production may result from a modulation of gene expression and reduction in mRNA expression and protein release for cytokines [32]. Macrolide therapy often appears to decrease the transcription of mRNA for a variety of cytokines [33].

The inflammatory cascade is composed of a network of cytokines and chemokines that modulate the initiation and progression of the inflammatory response. Pro-inflammatory cytokines include IL-1, IL-6, IL-8 and TNF-α. Modulation of these cytokines can decrease inflammation. Inhibition of pro-inflammatory cytokines and chemokine, particularly by 14-membered macrolides, including RXM, could help to prevent
phagocytic cells from entering the site of inflammation, and thereby attenuate undesired inflammatory processes [13,34, 35].

As these cytokines play an essential role in the immune-response to bacterial infections, the inhibition might have a negative effect on host defense in animals, which were treated with RXM. Therefore, these data suggest that the immune-modulator actions of RXM should be considered in the beneficial and detrimental aspects of inflammatory response.

In conclusion, the present study shows that roxithromycin can exert therapeutic anti-inflammatory effect independent of its antibacterial activity. Roxithromycin has anti-inflammatory activity in the acute and chronic models of inflammation tested in the doses of 5, 10 and 20mg/kg especially when used for long duration in the chronic model. Although the subcellular mechanisms underlying this activity are still unclear, the results reported in the literature and the present study agrees that macrolides affect several pathways of the inflammatory process, including the prostaglandins formation, migration of neutrophils and the production of proinflammatory cytokine TNF-α.

References


