Comparison of various macrolides on stimulation of human neutrophil degranulation in vitro

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Macrolide antibiotics are taken up and concentrated by host cells, particularly phagocytes, and are likely candidates to modify cell functions. In this study, we extended our previous work concerning the effect of three 14-membered-ring macrolides (dirithromycin, erythromycin and erythromycylamine) on human neutrophil exocytosis, and found that three other erythromycin A derivatives (roxithromycin, clarithromycin and the azalide, azithromycin) also triggered neutrophil degranulation in a time- and concentration-dependent manner. After 30 min of incubation, the correlation coefficients for concentration-dependence forroxithromycin were 0.885, 0.739 and 0.750 (P < 0.005) and for clarithromycin were 0.795, 0.599, 0.733 (P < 0.02), respectively, for lysozyme, β-glucuronidase and lactoferrin release. Although the underlying mechanism was not elucidated, these and previous data suggest that intracellular accumulation is a prerequisite. Furthermore, comparison of the characteristics of macrolide-induced exocytosis with those of exocytosis triggered by the synthetic chemotactic stimulus FMLP suggested that different mechanisms are involved. In keeping with this possibility, we showed that combined treatment (macrolides plus FMLP) resulted in totally additive exocytosis of azurophilic but not specific granules. The clinical relevance of our data remains to be ascertained.

Introduction

Macrolide antibiotics are strongly concentrated within phagocytes (reviewed in Labro 1993). Although the mechanisms underlying this cellular uptake are not fully clear, they may involve trapping by protonation inside acidic cell compartments (lysosomes and azurophilic granules) (Carlier, Zenebergh & Tulkens, 1987). The possibility that intragranular uptake interferes with phagocyte degranulation has rarely been explored (Engquist, Lundberg & Peterson, 1984; Carevic & Djokic, 1988; Anderson, 1989; Abdelghaffar, Mtairag & Labro, 1994). We have previously shown that three 14-membered-ring macrolides (dirithromycin, erythromycylamine and the prototype drug, erythromycin) stimulate human neutrophil degranulation in a time- and concentration-dependent manner (Abdelghaffar et al., 1994). In this study, we extended our investigations to various 14- and 16-membered-ring macrolides, by comparison with erythromycin. We also tested the azalide azithromycin, which is chemically derived

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from erythromycin and displays an outstanding ability to concentrate within human cells (Gladue et al., 1989). Part of this work was presented at the thirty-third Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, USA (Labro, Abdelghaffar & Bryskier, 1993).

**Materials and methods**

**Macrolides**

Erythromycin and roxithromycin (Roussel Uclaf, Romainville, France), clarithromycin (Laboratoire Abbott, Rungis, France), spiramycin (Rhône-Poulec-Rorer, Vitry sur Seine, France), josamycin (Pharmuka, Neuilly, France), rokitamycin (Laboratoire Pierre Fabre, Paris, France), oleandomycin and azithromycin (Pfizer, Orsay, France), dirithromycin (Eli Lilly, Indianapolis, USA) were kindly provided by the respective manufacturers. The drugs were dissolved (1 g/L) in dimethylsulphoxide (DMSO) and further diluted in Hanks buffered salt solution (HBSS-Institut Pasteur-) to the desired concentrations. DMSO was also diluted in HBSS to serve as a control.

**Human polymorphonuclear neutrophils**

Human neutrophils were obtained from heparinized venous blood of volunteers by Ficoll-Paque centrifugation followed by 2% dextran sedimentation and hypo-osmotic lysis of residual erythrocytes.

**Neutrophil viability**

Neutrophil viability was measured in all the experimental conditions used here (incubation with macrolides, DMSO or stimuli for up to 180 min, pH 7–9) by the release of lactate dehydrogenase (LDH), a cytoplasmic marker enzyme (Bergmeyer, 1963). In all conditions, LDH release represented less than 10% of total enzyme activity, indicating no alteration of cell viability compared to the control (8 ± 1.5%).

**Neutrophil degranulation**

Neutrophil degranulation was assessed by the release of three granular components: β-glucuronidase (located within azurophilic granules); lactoferrin (a marker of specific granules) and lysozyme (located in both granule subsets). β-glucuronidase release was assessed by the release of phenolphthalein from phenolphthalein glucuronic acid, monitored spectrophotometrically at 540 nm (Talalay, Fishman & Huggins, 1946). The release of lactoferrin was determined by an enzyme-linked immunosorbet assay with an anti-human lactoferrin antibody (Hetherington, Spitznagel & Quie, 1983); lysozyme release was measured in terms of the lysis of *Micrococcus lysodeikticus*, monitored spectrophotometrically at 450 nm (Litwack, 1955).

In standard experiments, neutrophils were incubated for 5–180 min in the presence of macrolides (1–100 mg/L) or formyl-methionyl-leucyl-phenylalanine (FMLP $5 \times 10^{-6} \text{M}$) or control DMSO solutions, and cytochalasin B (5 mg/L). After incubation, cells were centrifuged at 400g for 10 min, and enzyme activities were measured in the pellet and supernatant. Enzyme release was expressed as the percentage
of enzyme activity in the supernatant relative to that in the pellet plus supernatant. The positive control was activity in a cell lysate obtained by sonication (three 15-sec bursts at 30% power from a Fisher sonic dismembranator set at 4°C) in the presence of 0.1% Triton X-100, which induced optimal enzyme recovery (results not shown). Activity in the pellet plus supernatant did not differ from overall activity measured in the cell lysate. We also checked that the macrolides (up to 100 mg/L) did not directly modify the enzyme activities of a cell lysate. Experimental conditions included variations in the pH of the medium from 7 to 9 and omission of cytochalasin B.

**Effect of macrolides on neutrophil degranulation triggered by FMLP**

Neutrophils were incubated for 30 min in the presence of macrolides (100 mg/L) or DMSO and cytochalasin B, and further stimulated with FMLP (5 × 10⁻⁶ M). Enzyme release was measured as indicated above.

**Statistical analysis**

Results are expressed as the mean ± S.E.M. of n experiments performed with neutrophils from different human volunteers. Experiments with macrolides were paired with controls performed in the presence of the corresponding DMSO solution. Analysis of variance (ANOVA) test was used for multiple comparisons. Paired data were analyzed by using Student's t test for normal distributions. All statistical tests were performed with the Statworks program, version 1.2 1985 (Cricket software).

**Results**

**Effect of macrolides on human neutrophil degranulation**

At the concentration of 100 mg/L, roxithromycin, clarithromycin, azithromycin and erythromycin significantly triggered neutrophil exocytosis of both lysozyme (Table I) and β-glucuronidase (Table II) in a time-dependent manner. Although the effects of roxithromycin and clarithromycin appeared earlier, the overall amount of enzyme released did not significantly differ at the end of the 3 h incubation period among the four drugs. By contrast, neither the 16-membered-ring macrolides (josamycin and spiramycin) nor oleandomycin promoted neutrophil degranulation, even after a long incubation period. As already observed by us (Abdelghaffar et al., 1994) and other authors (Niessen et al., 1991), FMLP-induced degranulation was rapid, with maximal enzyme release after 10 min and no further change. Lactoferrin release by macrolide-treated neutrophils was analyzed at 30 and 180 min. Only roxithromycin and clarithromycin (100 mg/L) induced significant release of lactoferrin by neutrophils at 30 min (36 ± 5.0 and 26 ± 4.7%, P < 0.001 respectively for roxithromycin (15 experiments) and clarithromycin (12 experiments) vs 9 ± 1.5% for the control DMSO solution (15 experiments) and 51 ± 6.3% for FMLP (14 experiments). At 180 min, roxithromycin and clarithromycin (two experiments) promoted about 50% lactoferrin release by neutrophils, whereas azithromycin was slightly less effective (31 ± 5.1%; 4 experiments; P < 0.05 vs control DMSO—13 ± 0.8%). None of the other macrolides induced lactoferrin release, even after a 180 min incubation period (data not shown).
Table 1. Effect of macrolides (100 mg/L) on lysozyme release by neutrophils

<table>
<thead>
<tr>
<th>Macrolides</th>
<th>Incubation time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Roxithromycin</td>
<td></td>
</tr>
<tr>
<td>14 ± 2.7</td>
<td>(6)*</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td></td>
</tr>
<tr>
<td>16 ± 3.9</td>
<td>(6)*</td>
</tr>
<tr>
<td>Azithromycin</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>(1)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td></td>
</tr>
<tr>
<td>—</td>
<td>(1)</td>
</tr>
<tr>
<td>Oleandomycin</td>
<td></td>
</tr>
<tr>
<td>—</td>
<td>(1)</td>
</tr>
<tr>
<td>Spiramycin</td>
<td></td>
</tr>
<tr>
<td>—</td>
<td>(1)</td>
</tr>
<tr>
<td>Josamycin</td>
<td></td>
</tr>
<tr>
<td>—</td>
<td>(1)</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td>FMLP (5 × 10^-6 M)</td>
<td>—</td>
</tr>
<tr>
<td>DMSO (1%)</td>
<td>13 ± 2.1 (6)</td>
</tr>
</tbody>
</table>

*P < 0.01. Anova followed by Student's t-test (paired data) vs control DMSO solutions.

*Number of experiments is given in parentheses.
<table>
<thead>
<tr>
<th>Macrolides</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roxithromycin</td>
<td>7 ± 1.6 (4)*</td>
<td>7 ± 1.8 (6)</td>
<td>7 ± 2.4 (4)</td>
<td>19 ± 3.1* (9)</td>
<td>37 ± 5.8* (7)</td>
<td>48 ± 4.8* (8)</td>
<td>52 ± 7.2* (9)</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>6 ± 1.3 (4)</td>
<td>8 ± 1.6 (5)</td>
<td>9 ± 2.3 (4)</td>
<td>17 ± 2.3* (4)</td>
<td>30 ± 4.9* (5)</td>
<td>49 ± 4.7* (7)</td>
<td>52 ± 7.2* (8)</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>7 ± 2.3 (3)</td>
<td>—     (1)</td>
<td>12 ± 1.2 (3)</td>
<td>38 ± 5.5* (7)</td>
<td>44 ± 8.8* (4)</td>
<td>66 ± 6.5* (7)</td>
<td>—     (3)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>—     (1)</td>
<td>—     (1)</td>
<td>6 ± 4.9 (3)</td>
<td>16 ± 6.1 (2)</td>
<td>19 ± 4.3* (4)</td>
<td>34 ± 1.3* (3)</td>
<td>—     (3)</td>
</tr>
<tr>
<td>Oleandomycin</td>
<td>—     (1)</td>
<td>—     (1)</td>
<td>6 ± 2.1 (3)</td>
<td>5 ± 1.0 (2)</td>
<td>6 ± 1.0 (2)</td>
<td>8 ± 2.0 (2)</td>
<td>—     (3)</td>
</tr>
<tr>
<td>Spiramycin</td>
<td>—     (1)</td>
<td>—     (1)</td>
<td>4 ± 0.5 (3)</td>
<td>6 ± 1.0 (1)</td>
<td>11 ± 3.8 (1)</td>
<td>10 ± 3.8 (1)</td>
<td>—     (3)</td>
</tr>
<tr>
<td>Josamycin</td>
<td>—     (1)</td>
<td>—     (1)</td>
<td>5 ± 2.6 (3)</td>
<td>5 ± 2.6 (1)</td>
<td>9 ± 2.3 (1)</td>
<td>9 ± 2.3 (1)</td>
<td>—     (3)</td>
</tr>
<tr>
<td>Controls</td>
<td>—     (1)</td>
<td>12 ± 3.4* (3)</td>
<td>—     (1)</td>
<td>20 ± 1.9* (3)</td>
<td>17 ± 1.8* (3)</td>
<td>16 ± 2.7* (3)</td>
<td>20 ± 2.7* (3)</td>
</tr>
<tr>
<td>FMLP</td>
<td>—     (1)</td>
<td>12 ± 3.4* (3)</td>
<td>—     (1)</td>
<td>20 ± 1.9* (3)</td>
<td>17 ± 1.8* (3)</td>
<td>16 ± 2.7* (3)</td>
<td>20 ± 2.7* (3)</td>
</tr>
<tr>
<td>DMSO</td>
<td>5 ± 1.5 (4)</td>
<td>5 ± 1.2 (6)</td>
<td>4 ± 0.3 (4)</td>
<td>7 ± 1.9 (9)</td>
<td>5 ± 0.9 (7)</td>
<td>9 ± 2.8 (8)</td>
<td>10 ± 3.1 (9)</td>
</tr>
</tbody>
</table>

*P < 0.01 Anova followed by Student's t-test (paired data) vs control DMSO solutions.

*Number of experiments is given in parentheses.
Concentration-dependence of enzyme release

This was analyzed at 30 min with roxithromycin and clarithromycin and at 60 min with azithromycin (Figure 1A and B). By regression analysis, there was a good correlation between roxithromycin, clarithromycin and azithromycin concentrations and the release of lysozyme (roxithromycin, slope 0.29, \( r = 0.885, P < 0.001 \); clarithromycin, slope 0.25, \( r = 0.795, P < 0.001 \); azithromycin, slope 0.30, \( r = 0.945, P < 0.001 \)) and \( \beta \)-glucuronidase (roxithromycin, slope 0.22, \( r = 0.739, P < 0.01 \); clarithromycin, slope 0.16, \( r = 0.599, P < 0.01 \); azithromycin, slope 0.28, \( r = 0.804, P < 0.001 \)). The concentration-dependence of lactoferrin release by neutrophils incubated for 30 min in the presence of roxithromycin and clarithromycin was also significant (roxithromycin, slope 0.45, \( r = 0.750, P = 0.005 \); clarithromycin, slope 0.35, \( r = 0.733, P = 0.016 \)).

The kinetics of enzyme release was also analyzed with roxithromycin and azithromycin at a therapeutic concentration (achievable in tissues) of 10 mg/L (Figure 2). Both macrolides induced significant, time-dependent release of lysozyme and \( \beta \)-glucuronidase. By regression analysis, we found a close correlation between the incubation time and neutrophil exocytosis compared to control DMSO solutions (\( P < 0.05 \) azithromycin and \( P < 0.01 \) roxithromycin). After a 3 h incubation period, lysozyme and \( \beta \)-glucuronidase release was respectively 23 ± 2.2% (\( P = 0.039 \)) and 18 ± 3.5% (\( P = 0.032 \)), azithromycin, 6 and 7 experiments, and 31 ± 2.0% (\( P = 0.004 \)) and 23 ± 1.2% (\( P = 0.015 \)), roxithromycin, three experiments, vs control exocytosis in 0.1% DMSO (15 ± 1.1 and 9 ± 1.1, six and seven experiments).

Effect of pH on macrolide-induced degranulation

It is widely acknowledged that macrolide antibiotics enter cells better when the medium is alkaline (Labro, 1993). With the dibasic macrolides (dirithromycin and erythromycylamine), uptake measured at 30 min increases over a wide pH range up to pH 9 (Mtairag, Abdelghaffar & Labro, 1994). The uptake of other erythromycin A derivatives such as roxithromycin and clarithromycin has been reported to increase up to pH 8 (Carlier et al., 1987; Ishiguro et al., 1989) and Hand, King-Thompson & Holman (1987) have reported that maximal uptake of roxithromycin occurs at pH values around 8 and then decreases. We thus investigated whether a modification of the medium pH also affecting the degranulating effect of macrolides, as we had previously observed with dirithromycin and erythromycylamine (Abdelghaffar et al., 1994). Roxithromycin- and clarithromycin-induced exocytosis was little affected by the external pH (Figure 3) (Anova: roxithromycin: \( P = 0.062 \), lysozyme; \( P = 0.199 \), \( \beta \)-glucuronidase; clarithromycin: \( P = 0.119 \), lysozyme; \( P = 0.145 \), \( \beta \)-glucuronidase). By contrast, azithromycin-promoted degranulation (60 min incubation) was significantly increased over the pH range (Anova: \( P = 0.039 \)). This effect was similar to that observed with the other dibasic compound dirithromycin (60 min incubation), used as a control.

Effect of cytochalasin B on macrolide-induced exocytosis

Cytochalasin B, a fungal alkaloid which disrupts actin filaments, permits the degranulation of azurophilic granules in neutrophils stimulated with FMLP, and strongly enhances that of specific granules (Dewald et al., 1989; Niessen et al.,
Macrolides and neutrophil exocytosis

Figure 1. Correlation between macrolide concentration and enzyme release. (a) Lysozyme release: •, roxithromycin, 30 min, slope 0.29, r 0.885; □, clarithromycin, 30 min, slope 0.25, r 0.795; △, azithromycin, 60 min; slope 0.30, r 0.945. (b) β-glucuronidase release: •, roxithromycin, 30 min, slope 0.22, r 0.739; □, clarithromycin, 30 min, slope 0.16, r 0.599; △, azithromycin, 60 min; slope 0.28, r 0.804.

1991; Roos et al., 1993). We thus studied whether this agent also interfered with the degranulation process triggered by macrolides. Neutrophils were incubated (30–180 min) in the presence of macrolides (100 mg/L), DMSO or FMLP (5 × 10⁻⁶ M), with or without cytochalasin B. The stimulating effect of FMLP on lysozyme and β-glucuronidase release was completely abolished in the absence of cytochalasin B throughout the incubation period (Table III); by contrast, roxithromycin stimulation was significantly more rapid in the presence of this agent but still occurred and increased in a time-dependent manner in its absence (Table III). Similar data were obtained with clarithromycin (data not shown). This partial cytochalasin B-dependence of macrolide-induced exocytosis has also been observed with dirithromycin, erythromycin and erythromycin (Abdelghaffar et al., 1994).

Effect of macrolides on FMLP-stimulated neutrophil degranulation

As macrolide- and FMLP-promoted neutrophil exocytosis differ with regard to kinetics and cytochalasin B dependence, suggesting that the underlying transduction mechanism(s) or target granule subset differ, we studied whether the combination of
FMLP plus macrolides had an additive effect on neutrophil exocytosis. Neutrophils were incubated with roxithromycin (100 mg/L) or DMSO (1%) for 30 min and then stimulated with FMLP ($5 \times 10^{-6}$ M) for 10 min, before measurement of $\beta$-glucuronidase, lactoferrin and lysozyme release. Control (DMSO-treated and FMLP-stimulated) neutrophils released $19 \pm 2.1$ (22 experiments), $54 \pm 8.0$ (7 experiments) and $30 \pm 2.0\%$ (15 experiments) of the respective granule markers. Roxithromycin-treated and non-stimulated neutrophils released $25 \pm 2.4$, $42 \pm 7.9$ and $38 \pm 2.3\%$ of the granule markers. When roxithromycin-treated neutrophils were further stimulated with FMLP, there was a significant increase in $\beta$-glucuronidase release: $35 \pm 4.0$ ($P = 0.006$ vs FMLP alone and $P = 0.035$ vs roxithromycin alone) and lysozyme release: $50 \pm 2.5\%$ ($P = 0.006$ vs FMLP alone and $P = 0.001$ vs roxithromycin alone). Lactoferrin-induced degranulation by the drug combination ($67 \pm 6.9\%$) did not differ significantly from that triggered by each stimulus alone ($P = 0.063$). Similar results were obtained with clarithromycin (data not shown).

**Discussion**

We found that two 14-membered-ring macrolides, roxithromycin and clarithromycin and the azalide azithromycin, but not various 16-membered-ring macrolides or oleandomycin, promoted neutrophil exocytosis in a time- and concentration-dependent manner (Tables I, II and Figure 1). In a preliminary study (Abdelghaffar et al., 1994), we observed a similar effect with erythromycin, erythromycinylamine and dirithromycin, three 14-membered-ring macrolides. Although it is too early to propose a structure-activity relationship for this degranulating effect, it is noteworthy that all the compounds endowed with this property are chemically derived from erythromycin A.
Figure 3. Effect of pH on macrolide (100 mg/L)-induced enzyme release. (a) Lysozyme-release; (b) β-glucuronidase-release. Mean of four to nine experiments: 1% DMSO (○) and roxithromycin (○); two to six experiments: clarithromycin (□); three experiments azithromycin (■) and dirithromycin (▲). The incubation times were respectively 30 min: roxithromycin and clarithromycin and 60 min: DMSO, azithromycin and dirithromycin.

and thus possess similar carbohydrate components (L-cladinose and D-desosamine). It remains to be determined whether these sugars alone, or their structural combination with a lactone ring, confers this property.

The effect of macrolides on neutrophil degranulation has rarely been explored. Engquist, Lundberg & Petterson (1984) observed that neutrophils incubated for 30 min in the presence of erythromycin (125 mg/L) released significantly more proteases after FMLP stimulation than control cells, whereas erythromycin alone did not promote protease release. Anderson (1989) observed no changes in the exocytosis of neutrophils, regardless of FMLP stimulation, after 30 min of incubation in the presence
of roxithromycin or erythromycin at concentrations of <20 mg/L. By contrast, Carevic & Djokic (1988) have reported that incubation for 15 min with azithromycin and erythromycin (<10 mg/L) decreased the release of intragranular enzymes by immune-complex-stimulated neutrophils. Our data do not conflict with these results, as we observed that the neutrophil exocytosis induced by erythromycin A-derived macrolides is clearly dependent both on the concentration and on the incubation time, and that low concentrations of macrolides (10–25 mg/L) require a minimum of 2–3 h to be effective on exocytosis. Furthermore, neither Engquist et al. (1984) nor Carevic & Djokic (1988) used cytochalasin B, an agent which appears to favour macrolide-induced exocytosis (Table III). The apparent decrease in the external release of enzymes in the study by Carevic & Djokic (1988) could be because these authors used immune complexes as the stimulus, and that part of the degranulation could occur inside rather than outside the cells.

The mechanism underlying the degranulating effect of macrolides remains to be elucidated. Intracellular accumulation appears to be necessary, as suggested by the observation that experimental conditions which favour macrolide uptake also favour the degranulating effect. In particular, alkalinization of the medium significantly enhances the exocytosis stimulated by erythromycylamine and dirithromycin (Abdelghaffar et al., 1994), two dibasic macrolides whose uptake by neutrophils is extremely sensitive to the environmental pH (Mtairag et al., 1994). A similar effect was shown with azithromycin (Figure 2), which is also a dibasic macrolide. In the case of roxithromycin and clarithromycin, whose maximal uptake occurs around pH 8 (Hand et al., 1987; Ishiguro et al., 1989), we found no significant change in neutrophil exocytosis stimulated by these drugs when the pH exceeded 7.4 (Figure 2). A similar effect has been described with erythromycin A (Abdelghaffar et al., 1994): pH values above 7.4 did not modify β-glucuronidase release and only slightly affected lysozyme release.

### Table III. Effect of cytochalasin B on roxithromycin-induced exocytosis

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Cytochalasin B</th>
<th>Incubation time (min)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>DMSO</td>
<td>+</td>
<td>15 ± 4.8 (7)*</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>16 ± 5.6 (7)</td>
</tr>
<tr>
<td>Roxithromycin</td>
<td>+</td>
<td>41 ± 4.2 (7)</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>25 ± 4.1 (7)*</td>
</tr>
<tr>
<td>FMLP</td>
<td>+</td>
<td>30 ± 6.8 (4)</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>16 ± 3.7 (4)*</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>β-glucuronidase release (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
</tr>
<tr>
<td></td>
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<td>Roxithromycin</td>
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<tr>
<td></td>
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<tr>
<td>FMLP</td>
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<td></td>
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</tbody>
</table>

*Number of experiments is given in parentheses.
*P < 0.001.
*P < 0.05 Anova followed by Student's t-test (paired data).
Various authors have reported that drugs which induce intragranular alkalinization, such as monensin and Nigericin (two cationic ionophores) (Fittschen & Henson, 1991), chloroquine (an antimalarial weak base which is concentrated within neutrophil granules) (Fontagne et al., 1989), and Bafilomycin (a macrolide antibiotic which inhibits V-ATPase pumps responsible for intragranular acidification) (Bowman, Siebers & Altendorf, 1988; Swallow et al., 1991) also promote neutrophil degranulation. In the case of Nigericin and monensin, only the exocytosis of azurophilic granules was promoted and the authors proposed that intracellular alkalinization specifically triggered the release of this granule subset. However, there are no published data on the effect of macrolides on intragranular alkalinization. Furthermore, macrolides induce both azurophilic and specific granule exocytosis, as shown by the release of specific markers (respectively β-glucuronidase and lactoferrin). The hypothesis that intragranular accumulation of macrolides is responsible for their effects on neutrophil exocytosis is not supported either by the results obtained with dirithromycin (Abdelghaffar et al., 1994) and azithromycin (Tables I and II) compared with roxithromycin and clarithromycin. Although the former drugs are significantly more markedly trapped within neutrophil granules (Carlier et al., 1987; Gladue et al., 1989; Mtairag et al., 1995), they do not appear to be more effective in inducing neutrophil exocytosis. These data suggest that intragranular uptake is not the only factor underlying the triggering of neutrophil exocytosis, and that interaction of macrolides with a cytoplasmic target, possibly membranous, is the key event in macrolide-promoted exocytosis.

It is interesting to note that FMLP-induced exocytosis differs from that induced by macrolides with respect to kinetics, maximal enzyme release and dependence on cytochalasin B, suggesting that macrolides affect a transduction pathway differing from that stimulated by FMLP.

The clinical relevance of our findings also remains to be determined. We found that the degranulating effect could occur at therapeutic concentrations of the macrolides but required long incubation periods; a condition met in tissues. This property could be involved in one aspect of macrolide efficacy. These drugs are of recognized value for the treatment of infections caused by intracellular pathogens, and it is interesting to note that various microorganisms on which macrolides show intracellular bioactivity in vitro and in vivo, such as Toxoplasma, Mycobacterium avium, Mycobacterium leprae, Legionella spp., Chlamydia spp., reside in phagosomes by inhibiting phagolysosomal fusion (Miller et al., 1984; Bryskier, 1992; Bryskier et al., 1993; Stamm and Suchland, 1986). As macrolides are located in both the cytoplasm and the granular-lysosomal compartment (Labro, 1993), it is tempting to deduce that these drugs, by promoting phagolysosomal fusion, counteract the inhibitory effect of the microorganisms, thus exposing the latter to both the drugs and granular microbicidal components. Further investigations are required to verify this hypothesis.

Acknowledgement

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