Macrolide Antibiotics Protect against Endotoxin-Induced Vascular Leakage and Neutrophil Accumulation in Rat Trachea

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We studied the effects of macrolides on lipopolysaccharide (LPS)-induced airway inflammation in the rat tracheal mucosa. Erythromycin and roxithromycin dose dependently inhibited microvascular leakage and neutrophil recruitment induced by LPS. This inhibitory action on vascular permeability was abolished by neutrophil depletion.

There is increasing evidence that macrolide antibiotics modulate the functions of inflammatory cells such as polymorphonuclear leukocytes, lymphocytes, and macrophages (11) and directly affect airway secretory cell (5) and epithelial cell (14) functions. These effects are proposed to reflect the efficacy of macrolides in the treatment of inflammatory airway diseases (4, 9). In the present study, we characterized the effects of lipopolysaccharide (LPS) on vascular permeability and granulocyte recruitment in central airways and determined whether these changes were altered by erythromycin and roxithromycin (12).

Pathogen-free male Sprague-Dawley rats were divided into three treatment groups and orally given placebo, erythromycin (DAINABOT Co., Tokyo, Japan), or roxithromycin (Roussel Uclaf, Paris, France) at a daily dose of 1, 5, or 10 mg/kg of body weight for 1 week. The rats were anesthetized and injected with Monastral blue suspension (30 mg/kg; Sigma, St. Louis, Mo.) in the femoral vein (8). Immediately after the injection, LPS (Escherichia coli O26:B6, 1 mg/kg; Sigma) was administered intravenously to produce airway inflammatory reactions. At selected intervals, fixative (1% paraformaldehyde and saline in 50 mM phosphate buffer) was perfused through the left ventricle to wash Monastral blue pigments out of the vasculature. Then the trachea was removed, opened longitudinally, and incubated in the fixative for 3 h at room temperature. In a separate study, to deplete circulating granulocytes, cyclophosphamide (100 mg/kg) was injected intraperitoneally 5 days before the experiment. One day before the experiment, a second injection of cyclophosphamide (50 mg/kg) was given, causing a decrease in the peripheral neutrophils from (7.15 ± 0.83) × 109/μl to (0.036 ± 0.005) × 109/μl (n = 6; P < 0.001), and the effect of each macrolide (10 mg/kg) on LPS-induced microvascular leakage in neutropenic rats was assessed.

To stain myeloperoxidase-containing granulocytes, each tracheal section was incubated for 7 h at 4°C in a solution consisting of 25 mg of 3,3′-diaminobenzidine dissolved in 50 ml of 0.05 M Tris-HCl buffer at pH 7.6 and containing 0.5 ml of Triton X-100 and 0.5 ml of 1% hydrogen peroxide. Tracheal whole mounts were then prepared and neutrophils were counted (8). The magnitude of vascular permeability was assessed by using stereological point counting to determine the areal density of the blood vessels labeled with Monastral blue in the whole mount sections.

Data were expressed as means ± standard errors of the means. Statistical analysis was performed by using analysis of variance or Newman-Keuls multiple comparison test, and a P value of < 0.05 was considered significant.

As shown in Fig. 1, the number of neutrophils rapidly increased after LPS administration and reached maximal value at 3 h. A significant increase in the vascular permeability occurred 60 min after LPS administration and reached a plateau at 3 h.

Erythromycin reduced the area occupied by Monastral blue-labeled blood vessels in response to LPS from 7.8% ± 1.8% (n = 11) to 2.6% ± 0.5% at 5 mg/kg (n = 12; P < 0.05) and to 1.7% ± 0.5% at 10 mg/kg (n = 12; P < 0.01). Significant inhibition of the LPS-induced neutrophil recruitment was observed only with 10 mg of erythromycin per kg (318 ± 51 cells per mm² and n = 11 versus 20 ± 4 cells per mm² and n = 12; P < 0.001). Similar effects were observed with the roxithromycin-treated rats (Fig. 2). Neutrophil depletion per se decreased LPS-induced vascular leakage, and erythromycin and roxithromycin did not alter the effect of LPS in the neutropenic rats (Table 1).

These studies demonstrate that LPS increased neutrophil migration to the mucosal surface, which was followed by a corresponding increase in the magnitude of extravasation of Monastral blue from postcapillary venules in the rat trachea. Although the mechanism by which LPS increases tracheal vascular permeability is not completely understood, LPS may induce neutrophil activation and adherence of neutrophils to microvascular endothelial cells, which results in endothelial cell injury (7). We found that the accumulation of neutrophils coincided with the time of maximal response of vascular permeability and that neutrophil depletion attenuated the LPS-induced vascular leakage. Therefore, neutrophils may be

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<td>Erythromycin</td>
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<td>Roxithromycin</td>
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<td>Neutropenic rats</td>
<td>2.9 ± 0.3**</td>
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*Values are expressed as percent areal density of Monastral blue-labeled blood vessels in the tracheal whole mounts. Data are means ± standard errors of the means; n = 6 to 10. ** P < 0.01, significantly different from the corresponding values for normal rats.

* Each macrolide was administered at 10 mg/kg for 1 week.
FIG. 1. Time course of the effect of LPS on vascular permeability (upper panel) and neutrophil recruitment (lower panel) in rat tracheal whole mounts. LPS (1 mg/kg, solid circles) or its vehicle dimethyl sulfoxide (open circles) was intravenously administered at time zero. Vascular permeability and neutrophil recruitment were evaluated by measuring the areal density of Monastral blue-labeled blood vessels and counting the number of neutrophils, respectively, in the tracheal whole mounts. Values are expressed as means ± standard errors of the means; n = 8 to 12 for each point. *, P < 0.05; **, P < 0.01; ***, P < 0.001 (significantly different from the corresponding values for the vehicle alone).

FIG. 2. Effects of erythromycin (solid circles) and roxithromycin (solid triangles) on the LPS-induced increases in vascular permeability and neutrophil recruitment. The rats received either macrolide orally at a daily dose of 1.5, or 10 mg/kg for 1 week, and the areal density of Monastral blue-labeled blood vessels and the number of neutrophils were determined 3 h after LPS injection. Values are means ± standard errors of the means; n = 8 to 12 for each point. *, P < 0.05; **, P < 0.01; ***, P < 0.001 (significantly different from the corresponding values for LPS alone).

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REFERENCES