

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 $(7.15 \pm 0.83) \times 10^3$ to $(0.036 \pm 0.005) \times 10^3/\text{mm}^3$ ($n = 6$; $P < 0.001$), 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 \pm 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 $7.8\% \pm 1.8\%$ ($n = 11$) to $2.6\% \pm 0.5\%$ at 5 mg/kg ($n = 12$; $P < 0.05$) and to $1.7\% \pm 0.5\%$ 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 mm^2 and $n = 11$ versus 20 ± 4 cells per mm^2 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

TABLE 1. Effects of erythromycin and roxithromycin on LPS-induced vascular leakage in neutropenic rats^a

Macrolide ^b	Control rats	Effect of LPS in:		Neutropenic rats receiving macrolide
		Normal rats	Neutropenic rats	
Erythromycin	0.5 ± 0.3	8.0 ± 1.4	$3.1 \pm 0.2^{**}$	$3.4 \pm 0.3^{**}$
Roxithromycin	0.3 ± 0.2	7.5 ± 1.0	$3.2 \pm 0.4^{**}$	$2.9 \pm 0.3^{**}$

^a Values are expressed as percent areal density of Monastral blue-labeled blood vessels in the tracheal whole mounts. Data are means \pm standard errors of the means; $n = 6$ to 10. **, $P < 0.01$, significantly different from the corresponding values for normal rats.

^b Each macrolide was administered at 10 mg/kg for 1 week.

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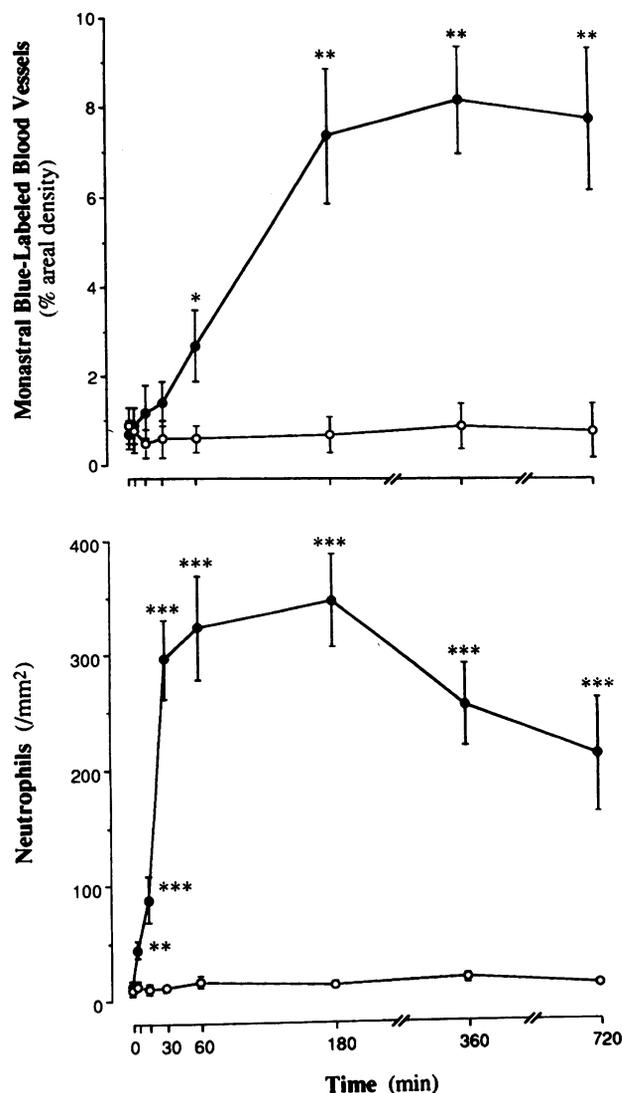


FIG. 1. Time course of the effect of LPS on vascular permeability (upper panel) and neutrophil recruitment (lower panel) in rat tracheal whole mounts. LPS (1 mg/kg, solid circles) or its vehicle dimethyl sulfoxide (open circles) was intravenously administered at time zero. Vascular permeability and neutrophil 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 \pm 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).

responsible for the LPS-induced increase in tracheal vascular permeability, in which activated neutrophils injure endothelium directly by the physical process of migration through vascular walls and/or indirectly via the release of vasoactive mediators (12).

Whether macrolide antibiotics alter neutrophil migration has been controversial in previous studies (3, 10), but we found that erythromycin and roxithromycin reduced LPS-induced neutrophil recruitment, as well as vascular leakage, in a dose-dependent fashion. Because LPS stimulates the expression of adhesion molecules for neutrophils (15) and the

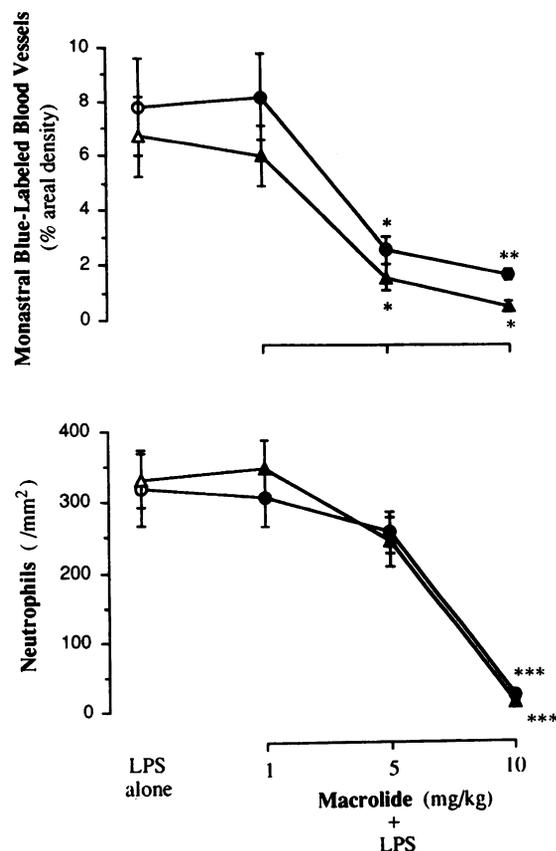


FIG. 2. Effects of erythromycin (solid circles) and roxithromycin (solid triangles) on the LPS-induced increases in vascular permeability and neutrophil recruitment. The rats received either macrolide orally at a daily dose of 1, 5, or 10 mg/kg for 1 week, and the areal density of Monastral blue-labeled blood vessels and the number of neutrophils were determined 3 h after LPS injection. Values are means \pm 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).

generation of chemotactic cytokines (2), these processes could also be involved in the protective effect of macrolides on neutrophil migration. The inhibition of vascular permeability by macrolides may not entirely be attributable to the inhibitory effect on neutrophil recruitment, because erythromycin and roxithromycin at 5 mg/kg each significantly decreased LPS-induced vascular leakage without altering the number of neutrophils present in the airway mucosa. Thus, macrolide antibiotics may also have acted on the events which occurred after neutrophil adhesion and/or directly on endothelial cells to inhibit the alterations in endothelial cell functions (6, 13), but the latter possibility is unlikely because the macrolides were no more effective in the neutropenic rats and because macrolides have been shown to inhibit the release of oxygen radicals (1) that cause much damage to the endothelial gap.

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REFERENCES

1. Anderson, R. 1989. Erythromycin and roxithromycin potentiate human neutrophil locomotion in vitro by inhibition of leukoattract-

- tant-activated superoxide generation and autooxidation. *J. Infect. Dis.* **159**:966–973.
2. **Billingham, M. E. J.** 1987. Cytokines as inflammatory mediators. *Br. Med. Bull.* **43**:350–370.
 3. **Fernandes, A. C., R. Anderson, A. J. Theron, G. Jooné, and C. E. J. Van Rensburg.** 1983. Enhancement of human polymorphonuclear leukocyte motility by erythromycin in vitro and in vivo. *S. Afr. Med. J.* **66**:173–177.
 4. **Fraschini, F., F. Scaglione, F. Ferrà, O. Marelli, P. C. Braga, and F. Teodori.** 1986. Evaluation of the immunostimulating activity of erythromycin in man. *Chemotherapy* **32**:286–290.
 5. **Goswami, S. K., S. Kivity, and Z. Marom.** 1990. Erythromycin inhibits respiratory glycoconjugate secretion from human airways in vitro. *Am. Rev. Respir. Dis.* **141**:72–78.
 6. **Harlar, L. M., L. A. Harker, M. A. Reider, C. M. Gajdusek, S. M. Schwartz, and G. E. Striker.** 1983. Lipopolysaccharide mediated bovine endothelial cell injury in vitro. *Lab. Invest.* **48**:269–274.
 7. **Janoff, A., and J. D. Zelig.** 1968. Vascular injury and lysis of basement membrane in vitro by neutral protease of human leukocytes. *Science* **161**:702–704.
 8. **McDonald, D. M.** 1988. Neurogenic inflammation in the rat trachea. I. Changes in venules, leucocytes, and epithelial cells. *J. Neurocytol.* **17**:583–603.
 9. **Miyatake, H., F. Taki, H. Taniguchi, R. Suzuki, K. Takagi, and T. Satake.** 1991. Erythromycin reduces the severity of bronchial hyperresponsiveness in asthma. *Chest* **97**:670–673.
 10. **Nelson, S., W. R. Summer, P. B. Terry, G. A. Warr, and G. J. Jakab.** 1987. Erythromycin-induced suppression of pulmonary antimicrobial defenses: a potential mechanism of superinfection in the lung. *Am. Rev. Respir. Dis.* **136**:1207–1212.
 11. **Roche, Y., M. A. Gougerot-Pocidalò, M. Fay, N. Forest, and J. J. Pocidalò.** 1986. Macrolides and immunity: effects of erythromycin and spiramycin on human mononuclear cell proliferation. *J. Antimicrob. Chemother.* **17**:195–203.
 12. **Sheppard, D., L. Scypinski, J. Horn, T. Gordon, and J. E. Thompson.** 1986. Granulocyte-mediated airway edema in guinea pigs. *J. Appl. Physiol.* **60**:1213–1220.
 13. **Svensjo, E., and G. J. Grega.** 1986. Evidence for endothelial cell-mediated regulation of macromolecular permeability by post-capillary venules. *Fed. Proc.* **45**:89–95.
 14. **Tamaoki, J., K. Isono, N. Sakai, T. Kanemura, and K. Konno.** 1992. Erythromycin inhibits C1 secretion across canine tracheal epithelial cells. *Eur. Respir. J.* **5**:234–238.
 15. **Winn, R. K., W. J. Mileski, N. L. Kovach, C. M. Doerschuk, C. L. Rice, and J. M. Harlan.** 1993. Role of protein synthesis and CD11/CD18 adhesion complex in neutrophil migration into the lung. *Exp. Lung Res.* **19**:221–235.