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Inhibition of Secretion of Interleukin-1α and Tumor Necrosis Factor Alpha by the Ketolide Antibiotic Telithromycin

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The antibiotic telithromycin was examined for its effect on secretion of interleukin-1α (IL-1α), IL-1β, IL-6, IL-10, and tumor necrosis factor alpha (TNF-α) by lipopolysaccharide (LPS)-stimulated monocytes of eight human donors. Secretion of each cytokine was significantly increased by LPS alone, whereas treatment with telithromycin significantly inhibited secretion of IL-1α and TNF-α but not secretion of IL-1β, IL-6, and IL-10. Telithromycin had immunomodulatory effects as a result of alteration of secretion of IL-1α and TNF-α by monocytes.

Many antibiotics, when administered in vitro or in vivo in animal models, have been shown to modulate the immune response and, in particular, the inflammatory response to bacterial infections (2, 14, 18). We and others have been interested in the study of the effects of antibiotics on the production of cytokines by human monocytes (9, 12, 15). In an in vitro system in which lipopolysaccharide (LPS)-stimulated human monocytes are treated with various antibiotics, we observed remarkable down-regulation of production of a number of cytokines with certain macrolides (9), quinupristin-dalfopristin (10), and quinolones (11). Telithromycin is a ketolide antibiotic derived from the macrolide clarithromycin by replacement of the cladinose at C-3 with a keto group (8). This alteration results in a more stable drug and decreases the level of induction of the macrolide-lincosamide-streptogramin B resistance phenotype. We report here that telithromycin has immunomodulatory activity as a result of the down-regulation of production of proinflammatory cytokines by human monocytes.

Telithromycin was obtained from Aventis Pharmaceuticals Inc. (Bridgewater, N.J.) and diluted in RPMI 1640 medium. LPS (*Escherichia coli* O26:B6) was purchased from Difco Laboratories, Detroit, Mich.

Blood was obtained by venipuncture from healthy donor volunteers. Peripheral blood mononuclear cells were obtained and cultured as described previously (12). Briefly, cells were separated on Ficoll-Paque and Percoll (Pharmacia Biotech AB, Uppsala, Sweden) density gradients. The monocyte-enriched cell fraction was collected, washed, and resuspended in RPMI 1640 medium (with 25 mM HEPES and L-glutamine; Mediatech, Inc., Herndon, Va.) containing 10% fetal bovine serum. Characterization of monocytes was with the Naphtol AS-D chloroacetate esterase and α-naphthyl acetate esterase kit (Sigma Diagnostics, St. Louis, Mo.) (19). At least 90% of the cells were identified as monocytes. These were seeded into 24-well plates (Costar Corporation, Cambridge, Mass.) at a density of 10^6 cells/ml (1 ml per well) and incubated in the presence of 100 ng of LPS per ml without or with 0.5, 1, 2, 5, or 10 μg of telithromycin per ml for 3, 6, or 24 h at 37°C in a 5% CO_2 incubator. Cell-free supernatants were recovered by centrifugation and stored at −20°C until assayed for interleukin-1α (IL-1α), IL-1β, IL-6, IL-10, and tumor necrosis factor alpha (TNF-α) by enzyme-linked immunosorbent assay with commercially available reagents from PharMingen (San Diego, Calif.) and Endogen (Woburn, Mass.). Quantification of each cytokine was based on a standard curve derived by linear dilution of the cytokine standards included in the respective kits. The detection limit for IL-1α and IL-10 was 8 pg/ml, that for IL-6 and TNF-α was 20 pg/ml, and that for IL-1β was 3.9 pg/ml. In reproducibility assays for each cytokine, the coefficient of variation was <12% in replicate assays with the same sample. Cytokine assays were performed in quadruplicate by using the supernatant samples or appropriate dilutions of the supernatants, as determined in preliminary studies. The toxicity of telithromycin for the purified monocytes was determined with the Cell Titer 96 kit [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay; Promega Corp., Madison, Wis.], as described previously (12). All values were expressed as means ± standard deviations. Welch's modified *t* test in computer software (InStat 2.0; GraphPad Software, San Diego, Calif.) was used to determine, for each donor, whether the difference in cytokine secretion between LPS-stimulated monocytes not treated or treated with telithromycin was statistically significant. A P value of ≤0.05 was considered statistically significant.

Although the amount of cytokines secreted by LPS-stimulated monocytes varied from individual to individual, for each of the eight donors it was always significantly higher ($P < 0.001$) than the amount secreted by non-LPS-stimulated monocytes. Tables 1 and 2 show the levels of secretion of IL-1α and TNF-α, respectively, by LPS-stimulated monocytes of each of the eight donors and the levels of inhibition of their secretion by telithromycin. Peak secretion of IL-1α was observed at 6 h for two donors and at 24 h for six donors. The percent decrease in the level of secretion of IL-1α from the peak value varied from 0 to 14% for monocytes treated with 1 μg of telithromycin per ml, from 6 to 63% for those treated with 5 μg of

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TABLE 1. Inhibition of IL-1α secretion in LPS-stimulated human monocytes treated with different concentrations of telithromycin

Donor	Time (h)	Amt (ng/ml) of IL-1α after treatment with the following concn (μg/ml) of telithromycin (% decrease[a]):			
		0	1	5	10
A	3	0.02 ± 0.003	0.02 ± 0.003	0.02 ± 0.002	0.01 ± 0.001
	6	0.13 ± 0.005	0.12 ± 0.003 (14)	0.06 ± 0.004[b] (56)	0.05 ± 0.003[b] (65)
	24	0.10 ± 0.002	0.10 ± 0.005	0.07 ± 0.005	0.07 ± 0.002
B	3	0.04 ± 0.002	0.06 ± 0.002	0.05 ± 0.002	0.05 ± 0.002
	6	0.20 ± 0.003	0.20 ± 0.007 (2)	0.08 ± 0.003[b] (63)	0.07 ± 0.003[b] (64)
	24	0.12 ± 0.004	0.12 ± 0.004	0.12 ± 0.008	0.07 ± 0.006
C	3	0.03 ± 0.005	0.02 ± 0.003	0.02 ± 0.002	0.02 ± 0.001
	6	0.17 ± 0.006	0.15 ± 0.009	0.10 ± 0.006	0.08 ± 0.002
	24	0.17 ± 0.004	0.18 ± 0.005 (0)	0.15 ± 0.005[b] (13)	0.14 ± 0.006[b] (15)
D	3	0.02 ± 0.001	0.02 ± 0.002	0.01 ± 0.003	0.01 ± 0.002
	6	0.05 ± 0.003	0.05 ± 0.002	0.04 ± 0.003	0.04 ± 0.003
	24	0.13 ± 0.005	0.15 ± 0.004 (0)	0.12 ± 0.004 (6)	0.10 ± 0.004[b] (20)
E	3	0.01 ± 0.001	0.02 ± 0.002	0.01 ± 0.002	0.01 ± 0.001
	6	0.13 ± 0.005	0.08 ± 0.004	0.07 ± 0.004	0.06 ± 0.004
	24	0.17 ± 0.006	0.17 ± 0.006 (0)	0.13 ± 0.009[b] (23)	0.10 ± 0.004[b] (38)
F	3	0.16 ± 0.005	0.10 ± 0.007	0.09 ± 0.004	0.06 ± 0.005
	6	0.13 ± 0.004	0.3 ± 0.005	0.17 ± 0.006	0.16 ± 0.005
	24	0.21 ± 0.006	0.12 ± 0.008 (7)	0.17 ± 0.003[b] (20)	0.15 ± 0.006[b] (28)
G	3	0.01 ± 0.001	0.02 ± 0.002	0.01 ± 0.002	0.01 ± 0.002
	6	0.04 ± 0.002	0.05 ± 0.005	0.04 ± 0.001	0.04 ± 0.002
	24	0.15 ± 0.004	0.14 ± 0.004 (10)	0.10 ± 0.002[b] (32)	0.09 ± 0.004[b] (39)
H	3	0.03 ± 0.002	0.02 ± 0.002	0.01 ± 0.001	0.01 ± 0.001
	6	0.05 ± 0.002	0.04 ± 0.005	0.04 ± 0.003	0.03 ± 0.001
	24	0.12 ± 0.002	0.11 ± 0.002 (11)	0.07 ± 0.0030[b] (43)	0.06 ± 0.005[b] (49)

[a] Percent decrease from the amount secreted by LPS-stimulated monocytes not treated with telithromycin.
[b] $P \leq 0.01$.

telithromycin per ml, and from 15 to 65% for those treated with 10 μg of telithromycin per ml.

Peak secretion of TNF-α was observed at 6 h for seven donors and at 3 h for one donor (Table 2). The percent decrease in the level of secretion of TNF-α from the peak value varied from 0 to 16% for monocytes treated with 1 μg of telithromycin per ml, from 15 to 52% for those treated with 5 μg of telithromycin per ml, and from 30 to 65% for those treated with 10 μg of telithromycin per ml. Treatment of the LPS-stimulated monocytes with 1 μg of telithromycin per ml did not result in significant inhibition of secretion of IL-1α or TNF-α; this concentration appeared to have a stimulatory effect on TNF-α secretion in two donors (donor D at 3 h and donor H at 6; Table 2). In contrast, treatment with 5 μg of telithromycin per ml significantly ($P \leq 0.01$) inhibited secretion of IL-1α in seven of eight donors, and treatment with 10 μg of telithromycin per ml significantly ($P \leq 0.01$) inhibited secretion of IL-1α in eight of eight donors ($P \leq 0.01$). Treatment of LPS-stimulated monocytes with 5 or 10 μg of telithromycin per ml significantly ($P \leq 0.01$) inhibited secretion of TNF-α in each of the eight donors.

Peak secretion of IL-1β, IL-6, and IL-10 by LPS-stimulated monocytes for each of the eight donors was at 24 h. However, their secretion was not significantly inhibited by treatment with any of the concentrations of telithromycin (data not shown). A lack of toxicity of telithromycin for the cultured monocytes was noted at all telithromycin concentrations used, as determined by the MTT assay. Previous studies also demonstrated that the concentration of LPS used to stimulate the monocytes in the present study was not toxic for these cells, which remained viable for at least an additional 24 h.

Our results revealed that stimulation of monocytes with LPS alone resulted in a significant increase in the level of accumulation of each cytokine in the supernatants of the cultured monocytes. Treatment with telithromycin resulted in a significant inhibitory effect on secretion of IL-1α and TNF-α but not on secretion of IL-1β, IL-6, or IL-10. The mean maximum concentrations of telithromycin in human serum following the administration of doses of 400, 800, or 1,600 mg have been reported to be 0.80, 1.9, and 4.07 mg/liter, respectively (16). The greatest immunomodulatory effect in our study in vitro was at 5 and 10 μg, levels not attainable by conventional dosing in humans. However, this may not rule out an in vivo effect of the drug on cytokine modulation by monocytes.

The down-regulatory effect on secretion of IL-1α is of interest since it is a major proinflammatory cytokine (5). In most studies, the biological activities of IL-1α and IL-1β are indistinguishable (3). However, recent work with a murine model of enteric inflammation caused by *Yersinia enterocolitica* revealed that IL-1α was the essential mediator of the intestinal inflammation and that IL-1β, TNF-α, or gamma interferon was not sufficient to induce the inflammatory changes (6).

TABLE 2. Inhibition of TNF-α secretion in LPS-stimulated human monocytes treated with different concentrations of telithromycin

Donor	Time (h)	Amt (ng/ml) of TNF-α after treatment with the following concn (μg/ml) of telithromycin (% decrease[a]):			
		0	1	5	10
A	3	1.35 ± 0.036	1.29 ± 0.023	1.20 ± 0.012	1.15 ± 0.012
	6	2.40 ± 0.027	2.43 ± 0.023 (0)	2.05 ± 0.018[b] (15)	1.63 ± 0.013[b] (33)
	24	0.67 ± 0.009	0.47 ± 0.010	0.35 ± 0.010	0.31 ± 0.009
B	3	2.11 ± 0.013	1.91 ± 0.015	1.65 ± 0.038	1.33 ± 0.021
	6	2.66 ± 0.034	2.43 ± 0.029 (7)	2.12 ± 0.037[b] (20)	1.76 ± 0.027[b] (35)
	24	0.87 ± 0.008	0.91 ± 0.008	0.70 ± 0.010	0.42 ± 0.006
C	3	0.63 ± 0.010	0.55 ± 0.011	0.53 ± 0.008	0.50 ± 0.007
	6	1.22 ± 0.036	1.12 ± 0.046 (7)	1.00 ± 0.032[b] (21)	0.82 ± 0.013[b] (36)
	24	0.12 ± 0.006	0.08 ± 0.003	0.06 ± 0.004	0.02 ± 0.003
D	3	4.40 ± 0.030	5.28 ± 0.052 (0)	3.49 ± 0.034[b] (22)	3.12 ± 0.019[b] (30)
	6	1.58 ± 0.018	1.41 ± 0.034	1.08 ± 0.046	1.05 ± 0.033
	24	0.83 ± 0.009	0.78 ± 0.006	0.61 ± 0.005	0.38 ± 0.008
E	3	0.77 ± 0.007	0.63 ± 0.005	0.50 ± 0.004	0.48 ± 0.007
	6	1.98 ± 0.042	1.87 ± 0.071 (9)	1.50 ± 0.054[b] (21)	1.17 ± 0.057[b] (42)
	24	0.60 ± 0.005	0.53 ± 0.006	0.47 ± 0.008	0.27 ± 0.006
F	3	1.35 ± 0.011	1.50 ± 0.013	1.39 ± 0.013	1.19 ± 0.016
	6	2.95 ± 0.035	2.74 ± 0.019 (8)	2.09 ± 0.012[b] (31)	1.67 ± 0.012[b] (45)
	24	1.10 ± 0.008	0.59 ± 0.007	0.41 ± 0.008	0.29 ± 0.002
G	3	0.71 ± 0.008	0.41 ± 0.007	0.29 ± 0.005	0.28 ± 0.004
	6	3.35 ± 0.034	2.85 ± 0.029 (16)	2.11 ± 0.037[b] (36)	1.42 ± 0.039[b] (58)
	24	1.49 ± 0.011	1.61 ± 0.009	1.42 ± 0.015	1.01 ± 0.011
H	3	0.86 ± 0.011	0.74 ± 0.009	0.69 ± 0.006	0.65 ± 0.003
	6	1.41 ± 0.052	1.54 ± 0.027 (0)	0.67 ± 0.004[b] (52)	0.50 ± 0.006[b] (65)
	24	0.45 ± 0.007	0.31 ± 0.004	0.24 ± 0.003	0.20 ± 0.006

[a] Percent decrease from the amount secreted by LPS-stimulated monocytes not treated with telithromycin.
[b] $P \leq 0.01$.

It is interesting to postulate that the differences in the action of telithromycin on secretion of IL-1α and IL-1β reflects differences in its activity on the cysteine proteases necessary for the cleavage of the pro forms to their mature forms necessary for secretion. Whereas pro-IL-1α requires cleavage to mature IL-1α by calpains before it can be secreted, pro-IL-1β is cleaved to its mature form by caspase-1 (IL-1β-converting enzyme) (4). Of interest in this regard are the findings of Bailly et al. (1), who noted an effect of ciprofloxacin on secretion of IL-1α and IL-1β that was the reverse of what we report here for telithromycin. Those investigators noted a posttranscriptional differential effect of ciprofloxacin on the synthesis of IL-1α and IL-1β. Ciprofloxacin reduced the total amount of IL-1β produced by LPS-stimulated human monocytes but not the total amount of IL-1α produced. In contrast, we previously noted that trovafloxacin significantly decreased the levels of secretion of both IL-1α and IL-1β (12).

We recently reported that trovafloxacin and ciprofloxacin, both of which down-regulate secretion of proinflammatory cytokines, improve the rates of survival among mice administered a lethal dose of *E. coli* LPS (11). Hirata et al. (7) have recently published similar observations from a study with clindamycin. Thus, the immunoregulatory activities of antibiotics may, in addition to their antimicrobial effects, have a protective effect against the destructive local inflammatory response in areas of infection (18). Because telithromycin is derived from a macrolide, it is of interest that the inhibitory effect of telithromycin was most pronounced for IL-1α and TNF-α. We previously demonstrated that azithromycin and clarithromycin also significantly inhibited the levels of secretion of IL-1α and TNF-α by LPS-stimulated human monocytes (9). Macrolides in particular have been shown to have immunomodulating properties of potential benefit in several noninfectious diseases as well as in inflammatory diseases caused by persisting microorganisms (13). In the latter case, it is unclear whether the effect of the antibiotic is through a direct action on the persistent pathogen or on the production of inflammatory mediators (14). However, during antibiotic therapy of acute bacterial infection due to a gram-negative bacterium, the production of inflammatory cytokines may be enhanced due to the release of endotoxin caused by the killing of the bacteria (17). These observations highlight the complexity of the interactions among antibiotics, infectious organisms, and cells of the immune system.

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