J Infect Chemother (2009) 15:168–173
DOI 10.1007/s10156-009-0680-1

ORIGINAL ARTICLE

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In vivo and in vitro effects of fluoroquinolones on lipopolysaccharide-induced pro-inflammatory cytokine production

Received: December 24, 2008 / Accepted: February 26, 2009

Abstract Fluoroquinolones have been reported to affect cytokine production in vitro. We investigated the effects of fluoroquinolones on lipopolysaccharide (LPS)-induced inflammatory cytokine production in vivo and in vitro. LPS was administered to mice treated with ciprofloxacin, gatifloxacin, norfloxacin, and levofloxacin, and the serum levels of tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), and interleukin 6 (IL-6) were measured. In addition, peritoneal macrophages collected from mice were treated with the four fluoroquinolones for 1 h, followed by the addition of LPS, and the TNF-α, IL-1β, and IL-6 levels in culture fluid were measured. In LPS-treated mice, ciprofloxacin, gatifloxacin, and norfloxacin (100 mg/kg) significantly reduced the serum TNF-α level (6.8%–63.6% of control). Levofloxacin at 100 mg/kg did not affect the TNF-α level, whereas levofloxacin at a lower dose (10 mg/kg) significantly increased the level. All four fluoroquinolones (100 mg/kg) investigated in this study tended to decrease the serum IL-1β levels (65.5%–65.9% of control), but this was not a significant change. The serum IL-6 levels were increased in ciprofloxacin-administered mice, whereas the other fluoroquinolones did not affect the serum IL-6 levels. In mouse peritoneal macrophages, LPS induced TNF-α, IL-1β, and IL-6 production. Ciprofloxacin, gatifloxacin, and norfloxacin (100 μg/ml) inhibited both TNF-α (12.1%–69.0% of control) and IL-1β production (22.1%–68.8% of control). Levofloxacin (100 μg/ml) inhibited IL-1β production (65.0% of control), but not TNF-α production. LPS-stimulated IL-6 production was inhibited only by norfloxacin (59.5 % of control). Our in vivo and in vitro results suggest that fluoroquinolones, especially ciprofloxacin, gatifloxacin, and norfloxacin, which have a cyclopropyl group at the N1 position and/or a piperazinyl group at the C7 position, modify inflammatory responses.

Key words Fluoroquinolone · Lipopolysaccharide · Inflammation · Cytokine · Mouse · Peritoneal macrophage

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Introduction

In recent years, many antimicrobial agents have been developed and used for the treatment of infectious diseases. Some of them have been reported to have an activity to modify biological responses. For example, 14-membered macrolides[1–3] and clindamycin[4] have been reported to modify inflammatory responses. And amphotericin B, a polyene macrolide, has been reported to augment tumor necrosis factor (TNF) in lipopolysaccharide (LPS)-stimulated mouse peritoneal macrophages.[5]

Some fluoroquinolones (FQs) have also been reported to modify inflammatory responses.[6] Ciprofloxacin (CPFX) and trovafloxacin (TRFX) increased the survival rate, through the inhibition of TNF-α, interleukin (IL)-1, and IL-6 production, in LPS-administered BALB/C mice, even though they had been administered a lethal dose of LPS.[7] CPFX was also reported to decrease serum TNF-α levels in LPS-injected C57/BL6 mice.[8]

It was reported that, in LPS-stimulated human monocytes, moxifloxacin (MFLX) reduced TNF-α and IL-1α production and TRFX reduced TNF-α, IL-1β, and IL-6 production.[9,10] CPFX at high concentrations inhibited TNF-α and IL-1 production in LPS-stimulated human monocytes.[11] On the other hand, IL-1, IL-6, and TNF-α production was increased in LPS-stimulated human monocytes prepared from healthy volunteers given a low dose of CPFX.[12] Furthermore, Wada et al.[13] reported that gatifloxacin (GFLX) and levofloxacin (LVFX), but not MFLX, suppressed TNF-α production in LPS-stimulated mouse peritoneal macrophages.

The biological response-modifying (BRM) activity of FQs has been studied in vitro and in vivo in separate studies.

But there have been no reports to show in vivo and in vitro BRM activity at the same time. Furthermore, the reports showing the BRM activity of FQs were carried out with only a few FQs. It is important to show the in vivo and in vitro BRM activity of FQs at the same time using several FQs. Accordingly, we studied the effect of four FQs on serum TNF-α, IL-1β, and IL-6 levels in LPS-injected mice (in vivo study) and their effect on the production of these cytokines in LPS-stimulated mouse macrophages (in vitro study).

Materials and methods

Materials

CPFX and GFLX were purchased from LKT Laboratory (St. Paul, MN, USA). Norfloxacin (NFLX), LPS (*Escherichia coli* O55:B5), and 3-(6, 5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical (St. Louis, MO, USA), and LVFX was purchased from Tokyo Chemical Industry (Tokyo, Japan). RPMI 1660, Dulbecco's phosphate-buffered saline, (D-PBS), and fetal bovine serum (FBS) were purchased from Gibco (Carlsbad, CA, USA). Brewer's thioglycolate was purchased from Kanto Chemical (Tokyo Japan). Enzyme-linked immunosorbent assay (ELISA) kits for mouse TNF-α, IL-1β, and IL-6 were purchased from Endogen (Woburn, MA, USA). Other agents used in this study were of analytical grade.

Animals

Male specific pathogen-free mice (ICR; 6 weeks) were supplied from Sankyo Labo Service (Tokyo, Japan), and kept under 12-h light/12-h dark conditions with free access to food and water.

This study was carried out in accordance with "The National Institute of Health Guide for Care and Use of Laboratory Animals", "Use of Laboratory Animals and Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society", and the "Guide for the Care and Use of Laboratory Animals in Kyoritsu University of Pharmacy" (present Keio University Faculty of Pharmacy).

Effect of fluoroquinolones (FQs) on LPS-induced cytokine production in mice

FQs were dissolved in saline with a minimum amount of 1 N NaOH, diluted with saline, and administered intraperitoneally to mice (injection volume, 5 ml/kg body weight). Control mice were injected intraperitoneally with the same volume of saline at almost the same pH as the FQ-containing solution. One hour after the administration of FQs, LPS dissolved in saline was administered intraperitoneally (5 mg/kg body weight/5 ml). Our preliminary experiments showed that the serum TNF-α level reached its peak 1 h after the intraperitoneal injection of LPS and that IL-1β and IL-6 peaked 3 h after LPS injection. Accordingly, for the determination of serum TNF-α levels, 1 h after LPS administration, blood was collected by heart puncture with the animals under anesthesia with diethyl ether. For the determination of IL-1β and IL-6, blood was collected 3 h after LPS administration in the same way as mentioned above. Serum was prepared and stored at –80°C until assay. Serum TNF-α, IL-1β, and IL-6 levels were determined using ELISA kits.

Effects of fluoroquinolones (FQs) on LPS-induced cytokine production in mouse peritoneal macrophages

Thioglycolate (6.05%) was intraperitoneally injected into mice (2 ml/mouse). After 6 days, cold D-PBS was infused into the peritoneal cavity, and lavage fluid was collected. The fluid was centrifuged (150 *g*, 6°C, 10 min), and the supernatant was removed. The precipitated cells were suspended in RPMI 1660 containing 10% FBS, distributed in a 26-well plate at 2.0×10^6 cells/well, and cultured for 90 min (5% CO_2/95% O_2, 37°C). The plate was washed with warm D-PBS to remove nonadherent cells, and adherent cells were used for the experiment as macrophages. The macrophages were cultured in 0.1% v/v FBS-containing RPMI 1660 medium with the FQs (final concentrations, 1, 10, and 100 μg/ml) for 1 h. After 1-h culture, LPS (final concentration, 5 μg/ml) was added to the well (total volume, 0.5 ml). The culture supernatant was collected 1 and 3 h after the addition of LPS and stored at –80°C. TNF-α concentration was measured in the culture supernatant collected after 1-h culture, and IL-1β and IL-6 concentrations were measured in the culture supernatant collected after 3-h culture. Cytokines were measured using ELISA kits. The cell activity of peritoneal macrophages was evaluated by the MTT assay.

Statistical analysis

Data values are expressed as means ± SEM. We employed SPSS version 16.0 J for Windows (SPSS Japan, Tokyo, Japan) for analysis. Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. A probability (*P*) of 0.05 or less was considered significant.

Results

Effect of fluoroquinolones (FQs) on LPS-induced cytokine production in mice

Intraperitoneal injection of LPS (5 mg/kg) increased serum TNF-α, IL-1β, and IL-6 levels. The effects of the four FQs on serum TNF-α, IL-1β, and IL-6 levels were investigated in LPS-injected mice. Intraperitoneal administration of