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Ciprofloxacin treatment *in vivo* increases the *ex vivo* capacity of lipopolysaccharide-stimulated human monocytes to produce IL-1, IL-6 and tumour necrosis factor-alpha

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SUMMARY

Because *in vitro* treatment with quinolones, at pharmacological concentrations, modifies lipopolysaccharide (LPS) induced production of cytokines by monocytes, we studied the effect of orally administered ciprofloxacin (25 mg/kg) on the capacity of peripheral blood monocytes of healthy volunteers to produce tumour necrosis factor-alpha (TNF-α), IL-1 activity, IL-1α, IL-1β and IL-6 *ex vivo* in response to endotoxin stimulation. After 7 days of ciprofloxacin, the extracellular and cellular production of TNF-α, the cellular production of IL-1 activity, the extracellular and cellular production of IL-1α, and the cellular production of IL-6 increased significantly. Seven days after the end of the treatment, values returned to basal levels or even lower. To our knowledge, this is the first demonstration that ciprofloxacin can modulate *in vivo* the capacity of human monocytes to react to an inflammatory stimulus such as endotoxin.

Keywords quinolone monocytes IL-1 IL-6 tumour necrosis factor

INTRODUCTION

IL-1α and IL-1β, IL-6 and tumour necrosis factor-alpha (TNF-α) are biologically active polypeptides produced by mononuclear phagocytes, notably in response to endotoxin, which play a critical role in the induction of inflammatory and immune responses (Bendtzen, 1988; Dinarello, 1988; Kishimoto, 1989; Larrick & Kunkel, 1988).

A wide range of anti-microbial agents, including the fluorinated piperazinyl substitute quinoline derivatives (quinolones), administered *in vivo* or *in vitro*, have been reported to modify both host immune and inflammatory responses (Hauser & Regminton, 1982; Anderson, 1985; Forsgren *et al.*, 1985) and cytokine production (Roche, Fay & Gougerot-Pocidalo, 1987, 1988; Bailly *et al.*, 1990a). We have shown that *in vitro*, the quinolones ciprofloxacin, pefloxacin and ofloxacin at pharmacological concentrations (above 25 μg/ml) significantly inhibit the production of IL-1β, IL-6 and TNF-α by human monocytes stimulated by lipopolysaccharide (LPS) (Bailly *et al.*, 1990a, 1990b). These findings could be of physiological relevance at sites of infection where quinolones may accumulate (Dalhoff & Weidner, 1984). In contrast, at concentrations usually attained in the serum after therapeutic administration (< 10 μg/ml), either no inhibitory effects on *in vitro* IL-1, IL-6 or TNF-α production by human monocytes were observed (Roche *et al.*, 1987; Bailly *et al.*, 1990a), or a slight enhancement of IL-1 activity production by mouse peritoneal macrophages (Petit *et al.*, 1987).

In order to determine the *in vivo* effect of a quinolone on cytokine production, we studied the effect of orally administered ciprofloxacin (25 mg/kg) on the capacity of peripheral blood monocytes from healthy human volunteers to produce IL-1α, IL-1β, IL-6 and TNF-α *ex-vivo* in response to endotoxin stimulation. Ciprofloxacin was chosen because, among the quinolones we tested, it was the most potent inhibitor of cytokine production in our *in vitro* studies.

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MATERIALS AND METHODS

Subjects

The study involved 10 healthy volunteers from our laboratory, aged 25–40 years and weighing 50–75 kg. They were free of acute or chronic disease, and took no medication during the 2 weeks preceding the trial. Eight subjects received ciprofloxacin (25 mg/kg) orally twice a day for 7 days, corresponding to a usual treatment. The other two subjects did not receive ciprofloxacin and served as controls in order to minimize technical artefacts and to assess the reproducibility of the culture conditions at days 0, 7 and 14.

Peripheral blood was collected the day before beginning the treatment (day 0), 2 h after the last administration of ciprofloxa-

cin (day 7) and 7 days after the end of the treatment (day 14). The study protocol was approved by the Ethics Committee of INSERM (Paris, France), and informed consent was obtained from each of the subjects.

Monocyte culture

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood using Ficoll–Isopaque (Eurobio, Paris, France). The cells were then washed and resuspended in culture medium (RPMI 1640; GIBCO, Grand Island, NY) supplemented with 2 mM glutamine, 100 mM pyruvate, 1% non-essential amino acids and 1% heat-inactivated fetal calf serum (FCS). Monocytes were counted using non-specific esterase staining and adjusted to a concentration of 5×10^5 monocytes/ml in RPMI 1640 with 1% FCS.

One millilitre per well of the cell suspension was incubated for 90 min at 37°C in multiwell plastic plates (24 × 17 mm; Falcon). Non-adherent cells were then removed by three vigorous washings with HBSS. More than 95% of the adherent cells were monocytes as determined by esterase staining.

Cytokine production

After adherence, the cells were washed and one ml of fresh serum-free culture medium was added to the monolayers with LPS (*Escherichia coli* 0.55:B5; Difco; 10 μg/ml). Cultures were incubated for 24 h at 37°C in a humidified atmosphere of 95% air and 5% CO_2. To determine extracellular cytokine concentrations, cell-free supernatants were collected, centrifuged at 800 *g* for 10 min and stored at −70°C until assay. To determine cell-associated cytokine concentrations, the cells remaining in the wells were covered with 1 ml of fresh medium and stored at −70°C. After thawing, the cells were sonicated before cytokine assay. In order to minimize between-assay differences, all the samples harvested at days 0, 7 and 14 were stored at −70°C and assayed on the same day. Cytokine concentrations did not change in day 0, day 7 and day 14 samples taken from the two untreated control subjects (data not shown), and were within the range of values obtained in the day 0 samples from the treated subjects.

Cytokine assays

IL-1 activity. Extracellular and cell-associated IL-1 activity were determined in terms of the capacity to stimulate thymocyte proliferation in the presence of submitogenic concentrations of phytohaemagglutinin (PHA) as previously described (Scala & Oppenheim, 1983). It was shown that addition of neutralizing specific anti-IL-1 antibodies inhibited the thymocyte proliferation (>90%) in our assay conditions. Results are expressed as ct/min ($\times 10^{-3}$) of ^{3}H-thymidine incorporated after 72 h of culture.

IL-1α and IL-1β. IL-1α and IL-1β concentrations in supernatants and lysates were determined using a specific ELISA as previously described (Ferrua *et al.*, 1988). This method recognizes both mature and the precursor form of each IL-1 species with a detection limit of 10–20 pg/ml. Results are expressed as ng per 5×10^5 cells.

IL-6. Extracellular and cell-associated IL-6 concentrations were measured using an ELISA with a detection limit of 0·0153 ng/ml (IL-6; Genzyme). Results are expressed as ng per 5×10^5 cells.

TNF-α. Extracellular and cell-associated TNF-α concentrations were measured using an IRMA with a detection limit of 15 pg/ml (Medgenix, Diagnostics Pasteur). Results are expressed as ng per 5×10^5 cells.

Statistical analysis

Data were analysed using Wilcoxon's paired *t*-test. Values are expressed as mean ± s.e.m.

RESULTS

TNF-α production

Table 1 shows that both extracellular and cell-associated TNF-α production by LPS-stimulated monocytes were significantly higher after 7 days of ciprofloxacin (day 7) than before its administration (day 0). In contrast, 7 days after the end of the treatment (day 14) extracellular TNF-α production fell below the day 0 values, and cell-associated TNF-α levels returned to baseline. TNF-α levels were always below the detection limit in cell lysates and supernatants of non-stimulated monocytes (data not shown).

IL-1 production

Extracellular and cell-associated IL-1 activity were measured in the same samples as those used to measure TNF-α levels. Extracellular IL-1 activity produced by LPS-stimulated human monocytes was higher at day 7 than at day 0 (Table 1). At day 14, cell-associated IL-1 activity had decreased compared with day 7. Ciprofloxacin did not induce IL-1 activity in non-stimulated monocytes.

Because the thymocyte proliferation assay is not strictly specific for IL-1 activity and because IL-1 activity is due to both IL-1α and IL-1β molecules, we used a specific ELISA to measure the proportion of each IL-1 species in each sample. Table 1 shows that extracellular and cell-associated IL-1α production were enhanced at day 7 compared with day 0. At day 14 (7 days after the end of the treatment), IL-1α production had returned to values similar to those at day 0. Extracellular and cell-associated IL-1β production did not increase significantly at day 7, but cell-associated IL-1β production was decreased at day 14 compared with day 7.

IL-6 production

Cell-associated IL-6 production increased between day 0 and day 7, and at day 14, cell-associated IL-6 levels returned to basal values (day 0), whereas extracellular IL-6 concentrations were significantly lower than at day 0 (Table 1).

DISCUSSION

Our results show that the oral administration of the quinoline derivative antibiotic ciprofloxacin to healthy volunteers, at 25 mg/kg body weight twice daily for 7 days, induces significant modifications of monocyte capacity to produce IL-1, IL-6 and TNF-α in response to *ex vivo* endotoxin stimulation. To our knowledge, this is the first demonstration that ciprofloxacin can modulate *in vivo* the capacity of human monocytes to react to an inflammatory stimulus such as endotoxin.

As shown in Table 1, extracellular and cell-associated production of the cytokines studied tended to increase between day 0 and day 7, although only extracellular and cell-associated

Table 1. Effects of ciprofloxacin on TNF, IL-1α, IL-1β and IL-6 production

	Cytokine levels*			Statistical analysis†		
	Day 0	Day 7	Day 14	Days 0–7	Days 7–14	Days 0–14
TNF-α						
Extracellular	1·84±0·39	2·42±0·46	1·32±0·29	$P<0·05$	$P<0·01$	$P<0·05$
Cell-associated	0·27±0·03	0·37±0·02	0·23±0·06	$P<0·01$	NS	NS
IL-1 activity						
Extracellular	13631±1906	19431±2913	16558±3969	$P<0·05$	NS	NS
Cell-associated	15095±1212	19703±2147	10896±1583	NS	$P<0·05$	NS
IL-1α						
Extracellular	0·22±0·03	0·28±0·03	0·23±0·06	$P<0·05$	NS	NS
Cell-associated	1·01±0·08	1·43±0·12	0·82±0·23	$P<0·05$	$P<0·05$	NS
IL-1β						
Extracellular	0·84±0·13	0·94±0·09	0·77±0·12	NS	NS	NS
Cell-associated	6·63±0·33	7·87±0·45	5·96±0·45	NS	$P<0·05$	NS
IL-6						
Extracellular	5·14±0·28	5·42±0·54	3·87±0·62	NS	$P<0·05$	$P<0·05$
Cell-associated	0·19±0·02	0·33±0·04	0·19±0·04	$P<0·05$	$P<0·05$	NS

* Monocytes were isolated from peripheral blood collected the day before the treatment (day 0), 2 h after the last administration of ciprofloxacin (day 7) and 7 days after the end of the treatment (day 14) and then stimulated with LPS (10 μg/ml) for 18 h. The supernatants and cell lysates were assayed for extracellular and cell-associated cytokine levels, using an IRMA for TNF-α, the thymocyte proliferation test at the 1/64 dilution for IL-1 activity, and specific ELISAs for IL-1α, IL-1β and IL-6. Results are expressed as ct/min of ^{3}H-thymidine incorporated for IL-1 activity and as ng/5 × 10^5 cells for TNFα, IL-1α, IL-1β and IL-6 and are the mean ± s.e.m. of eight samples assayed in duplicate.

† Data were analysed using Wilcoxon's paired *t*-test.

TNF-α and IL-1α productions and extracellular IL-1 activity and cell-associated IL-6 productions were significantly enhanced after 7 days' administration of ciprofloxacin (day 7). The increases in the other cytokines were at the limit of significance.

At day 14, i.e. 7 days after the end of the treatment, the capacity for cytokine production decreased compared with day 7, showing that the elevation observed at day 7 was indeed related to ciprofloxacin treatment. Extracellular TNF-α and IL-6 production were even significantly lower at day 14 than at day 0.

To minimize technical artefacts, monocytes from two healthy control subjects who did not receive ciprofloxacin were examined concomitantly with those from the ciprofloxacin-treated volunteers. No increase in cytokine production was observed at any time (data not shown).

Oral administration of ciprofloxacin to healthy volunteers for 7 days thus induced a transitory enhancement of the *ex vivo* response of monocytes to LPS in terms of IL-1, IL-6 and TNF-α production, while spontaneous production of these cytokines was not modified. It has been shown that following the administration of ciprofloxacin at the dose used here, i.e. 25 mg/kg twice daily, the mean serum peak concentration is between 3·5 and 4·5 μg/ml after the first dose and the half-life is 4–6·5 h; after 7 days' administration these values are slightly increased (Gonzalez *et al.*, 1984; Thadepalli *et al.*, 1988). In a previous study, we found that when added *in vitro* at similar concentrations, ciprofloxacin did not significantly alter IL-1, TNF-α or IL-6 production by human monocytes incubated with LPS for 24 h (Roche *et al.*, 1987; Bailly *et al.*, 1990a). Another study showed that ciprofloxacin (1–5 μg/ml) enhanced, *in vitro*, IL-1 activity produced by mouse peritoneal macrophages (Petit *et al.*, 1987; Stunkel, Hewlett & Schumberger, 1988). These results could suggest that *in vivo*, cellular interactions could modify monocytic reactivity to a secondary challenge by LPS. Whatever the mechanism involved, our findings indicate that the oral administration of ciprofloxacin to healthy volunteers can modulate the reactivity of monocytes to an inflammatory stimulus such as endotoxin in terms of IL-1, IL-6, and TNF-α production.

TNF-α, IL-6 and IL-1 are essential transmitters of cell-to-cell signals in both physiological and pathological immune processes and inflammation. They share a number of activities and have been found to synergize. Their physiological role in the defence against invasive pathogens is illustrated by their protective effect against experimental bacterial infections *in vivo* using low concentrations of recombinant cytokines (Urbaschek & Urbaschek, 1987; Bendtzen, 1988; Havell, 1989). Such observations have led to the concept that under normal conditions these cytokines act as essential signals in the development of appropriate defences against pathogens. However, exaggerated or prolonged release can lead to multi-organ failure or cachexia (Tracey *et al.*, 1986; Beutler, 1988). In our study the ciprofloxacin-induced increase in IL-1, TNF-α and IL-6 production by monocytes from peripheral blood, although statistically significant, was limited. Furthermore, after the end

of the treatment, levels returned to day 0 levels or even lower.

The complexity of the cytokine effects as well as the complexity of the interaction between host immune system and antibiotics (according to the pharmacokinetics of the antibiotic in the tissues and their infectious status) requires further studies to determine possible beneficial consequences on the course of infectious and inflammatory diseases.

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