Ciprofloxacin and Pefloxacin Suppress the Inflammatory Response in Rats

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It has been known that ciprofloxacin augments the humoral and cellular immune systems. The aim of the present study was to determine whether these properties affect the inflammatory response. Adult male rats, ciprofloxacin and pefloxacin were used. The healty rats were treated orally with an equal volumes of ciprofloxacin (50 mg/kg) pefloxacin (50 mg/kg) or distilled water for 10 days. Their effects on the inflammatory response were investigated by testing the formation of formaline-induced edema. The effects of two quinolones were also evaluated on gastic mucus secretion by using the Alcian blue dye binding method and haematological parameters. Ciprofloxacin and pefloxacin showed a significant antiinflammatory activity and decreased the white blood cell count (WBC). However, there was no significant difference in the other haematological parameters. These two quinolones stimulated gastric mucus secretions, but these increases were not statistically significant. These findings suggest that ciprofloxacin and pefloxacin (at 50 mg/kg *p.o.* doses) possess antiinflammatory activities and are well-tolerated orally. Further detailed investigations are needed to clarify the mechanisms of their antiinflammatory activities

Key words —— ciprofloxacin, pefloxacin, gastric mucus secretion, antiinflammatory activity, haematological parameters

INTRODUCTION

Local inflammatory response is manifested with the classical signs of inflammation-such as erythema, swelling, and pain-while systemic inflammatory response is manifested with the changes in physiological and haematological parameters due to bone marrow activation.

It has been known that some antibacterial agents have various effects on the immune system.[1)] It has been demonstrated that macrolides attenuate carrageenin-induced rat paw edema.[2)] Recently it has been shown that clarithromycin supressed both the systemic and local inflammatory response in Guinea pigs after surgical trauma.[3)] Ciprofloxacin is also well-known to have a positive immunomodulating effect.[4)] In the present study, we investigated the effects of two commonly used fluoroquinolones, ciprofloxacin and pefloxacin on experimental inflammation in rats. Experimental inflammation was achieved by using the formaline-induced hind paw edema method. Additionally, the effects of these drugs on haematological parameters and gastric mucus secretion were also evaluated.

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MATERIALS AND METHODS

Male albino rats (200–250 g) were used in the present study. Food and water were avaliable ad libitum. Food was removed 16 hr before the last day. All the rats used in the following experiments were subject to the Guiding Principles for the Care and Use of Laboratory Animals and the Recommendations from the Declaration of Helsinki. They were randomly housed eight per wire-mesh cage for at least 1 week before starting the experiments and a 12 hr/12 hr light/dark cycle was maintained. The first group received distilled water only. The second group (control) received water and theapplied inflammatory procedure. The others groups received either pefloxacin (50 mg/kg) or ciprofloxacin (50 mg/kg) and the applied inflammatory procedure.

Table 1. The Inhibitory Effects of Ciprofloxacin and Pefloxacin on Paw Edema (Paw Diameter mm)

Treatment	Initial	10th day	Difference	% edema	% edema inhibition
Control	5.63 ± 0.12	8.59 ± 0.39	2.96 ± 0.33	100	—
Ciprofloxacin (50 mg/kg)	5.28 ± 0.07	6.00 ± 0.11	0.68 ± 0.10*	22.9	77
Pefloxacin (50 mg/kg)	5.35 ± 0.07	5.88 ± 0.18	0.56 ± 0.16*	18.9	81

* $p < 0.01$ as compared with control.

Formation of the Inflammatory Response —— The hind-paw edema method[5] was performed by injecting 0.1 ml of a 2% formaldehyde solution to the back-left paw of each rat regardless of weight on the first and third days of the experiment. Then water, ciprofloxacin (50 mg/kg/day), or pefloxacin (50 mg/kg/day) were orally given to the animals for 10 days by using a polyethylene cathether. The size of the hind paws was measured daily and was compared with the controls. The percentage of edema and percentage of edema inhibition were calculated as shown below. (Control edema was assumed to be 100%.[6]

% edema = $N^1 \times 100 / N$

% edema inhibition = $(N - N^1) / N \times 100$

N: is the edema value of the control group (calculated by substracting the paw diameter measured on the 10th day from the initial paw-diameter measurement).

N^1: is the edema value of the experimental group.

Monitoring of Haematological Parameters —— The rats were anesthetized byusing inhalational ether each time a blood sample was taken by intracardiac puncture. A 1 ml aliquot of blood was drawn from each animal at day 0 and at day 10. The blood was put into heparinized tubes and haematological parameters were measured using a Coulter automated analyzer.

Measurement of Gastric Mucus —— On the last day of the experiment, the animals were deprived of food over-night but were allowed free access to water. Animals were humanely killed by using an overdose of ether. Each animal's stomach was removed immediately, and the gastric lumen was rinsed with 10 ml of ice-cold 0.25 mol/l sucrose. The mucus content of the glandular part of the stomach was determined spectrophotometrically at 605 nm by the Alcian blue dye binding method.[7,8] The results were expressed as μg of Alcian blue adhered per g of glandular stomach tissue.

Statistical Analysis —— Student's *t*-test was used for statistical analysis, and $p < 0.05$ was regarded as statistically significant. Results were expressed as the mean ± S.E.M.

Table 2. The Effects of Ciprofloxacin and Pefloxacin on Gastric Mucus Secretion

Groups	Gastric mucus secretion (μg/g)
Normal	825.0 ± 45.3
Control	537.1 ± 52.3*
Ciprofloxacin (50 mg/kg)	625.7 ± 42.3*
Pefloxacin (50 mg/kg)	638.6 ± 67.07*

*$p < 0.05$ as compared with normal.

RESULTS

As indicated in Table 1, ciprofloxacin and pefloxacin significantly reduced the animal's inflammatory response when they were given at 50 mg/kg/day for 10 days ($p < 0.01$). The inflammatory procedure significantly reduced the gastric mucus content ($p < 0.05$) (Table 2). Ciprofloxacin and pefloxacin insignificantly increased the gastric mucus content. The experimental inflammatory procedure caused a significant increase in the red blood cell (RBC) count, haemoglobin (Hgb) concentration, and hematocrit (Htc) levels, while these two quinolones reduced the white blood cell count (WBC). However,there was no significant change in the other haematological parameters (Table 3).

DISCUSSION

The results of the present study demonstrate that ciprofloxacin and pefloxacin have an inhibitory effect on the inflammatory response when they were given for 10 days. This particular timing of the treatment was chosen because it relates to the usual time of antimicrobial therapy in medical practice (1–

Table 3. Haematological Parameters of Rats Treated with Ciprofloxacin (50 mg/kg) or Pefloxacin (50 mg/kg) or Saline before and after Inflammatory Process

Parameter	Day	Control (Saline)	Ciprofloxacin (50 mg/kg)	Pefloxacin (50 mg/kg)
WBC	0	17.08 ± 2.86	18.06 ± 0.98	23.98 ± 3.45
($\times 10^3/\mu$l)	10	16.95 ± 2.01	10.58 ± 1.25*	12.93 ± 1.83*
RBC	0	8.05 ± 0.38	7.50 ± 0.25	8.40 ± 0.49
($\times 10^3/\mu$l)	10	10.16 ± 0.56*	8.19 ± 0.39	7.81 ± 0.50
Hgb	0	13.73 ± 0.49	13.45 ± 0.42	15.20 ± 0.87
(g/dl)	10	17.28 ± 1.08*	14.48 ± 0.61	13.68 ± 0.63
Hct	0	43.13 ± 1.70	44.13 ± 3.17	46.55 ± 2.99
(%)	10	54.90 ± 2.42*	45.83 ± 2.13	41.83 ± 2.57
Mean Corpuscular Volume (MCV)	0	53.65 ± 0.78	55.5 ± 0.97	54.03 ± 0.59
(fl)	10	54.28 ± 0.84	55.98 ± 0.88	53.03 ± 0.73
Mean Corpuscular Haemoglobin (MCH)	0	17.09 ± 0.47	17.95 ± 0.22	18.40 ± 0.50
(pg)	10	16.99 ± 0.22	17.71 ± 0.49	17.63 ± 0.37
Mean Corpuscular Haemoglobin Concentration (MCHC)	0	31.85 ± 0.53	32.36 ± 0.50	33.99 ± 0.57
(g/dl)	10	31.41 ± 0.71	31.63 ± 0.55	33.19 ± 0.44
Platelet Count (Plt)	0	782 ± 76	803 ± 78	815 ± 40
($\times 10^3$/ml)	10	1048 ± 53	1008 ± 60	872 ± 44

*$p < 0.05$ *vs.* first day. n : 8.

2 weeks). Doses used in this study were calculated asmilograms per kilogram of body-weight, base on the amounts used in human therapy.[9)]

Over 30 years ago, some investigators started reporting the effects of antibiotics on the immune response.[10,11)] It was reported that macrolides had an immunomodulatory effect[11,12)] and attenuated an increase in rat paw volume induced by injecting carregenin into the paws.[2)] It was also demostrated that ciprofloxacin increases the humoral immune responses in a dose–dependent manner.[4)] It is generally thought that drugs that positively influence the immune system probably possess antiinflammatory activities. Since ciprofloxacin has a positive immunomodulating effect, it would be of both interest and practical value if quinolone antibiotics effected the inflammatory response.

Ciprofloxacin and pefloxacin were well-tolerated antibiotics at the doses used this study. Whereas the inflammatory procedure decreased the level of gastric mucus secretion, the local inflammation may be a stressful procedure. Ciprofloxacin and pefloxacin may reduce the stress of the inflammatory procedure by decreasing the classical signs of inflammation, such as erytheme, edema, and pain. Additionally, there was a trend towards a higher total RBC count, Hgb concentration, and Htc level in the control group. In the groups that were given ciprofloxacin and pefloxacin, there was not a significant difference in these parameters, but a decrease was observed in the WBC. Quinolone-induced leucopenia[13)] may play a role in the last adverse effect. Any significant difference with quinolones was not observed in the other haematological parameters.

To summarize these experiments, we showed that ciprofloxacin and pefloxacin are well-tolerated antimicrobial agents that inhibit the inflammatory response. However, further more- detailed experiments are needed to clarify the precise mechanism of their inflammatory activities.

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