Therapeutic effects of a new quinolone, DU-6859a, on polymicrobial infections in a newly designed model of rat uterine endometritis

Hiroshige Mikamo*, K yoko Kawazoe, Y asumasa Sato, K oji Izumi and Teruhiko Tamaya

Department of Obstetrics and Gynaecology, School of Medicine, Gifu University, 40, Tsukasa-machi, Gifu City, Gifu 500, Japan

We evaluated the efficacy of a new quinolone, DU-6859a, using a new model of rat uterine endometritis. Rats were infected with mixed inocula of Escherichia coli and Bacteroides fragilis. The MICs of DU-6859a and levofloxacin against E. coli were 0.025 and 0.05 mg/L, respectively; those against B. fragilis were 0.20 and 0.39 mg/L, respectively. Immediately after inoculating $10^5$ cfu/rat of each organism, DU-6859a or levofloxacin (20 mg/kg po bid or tid, respectively, for 3 days) was administered and compared with the untreated group. The viable cell counts of E. coli and B. fragilis in the DU-6859a- and levofloxacin-treated groups were significantly lower than those in the untreated group. These results suggest that DU-6859a would be useful for treating polymicrobial infections in uterine endometritis.

**Introduction**

New fluoroquinolones have been widely used to treat various infections, because of their broad antibacterial spectra, bactericidal activities and good pharmacokinetics. DU-6859a is a new fluoroquinolone with the chemical structure (–)-7-(7S)-amino-5-azaspiro(2,4)heptan-5-yl-8-chloro-6-fluoro-1-(1,2R)-cis-2-fluoro-1-cyclopropyl-1,4-dihydro-4-oxoquinolone-3-carboxylic acid, which was initially synthesized by Daiichi Pharmaceutical Co. Ltd, Tokyo, Japan. DU-6859a displays relatively potent in-vitro activity compared with related quinolones.

Polymicrobial infections caused by aerobes and anaerobes are commonly seen in the female genital tract. We therefore evaluated the efficacy of two quinolones, DU-6859a and levofloxacin, using a new model of rat uterine endometritis caused by organisms isolated from human endometritis. This is the first report of the in-vivo activity of DU-6859a.

**Materials and methods**

**Animals**

Female Sprague-Dawley rats (specific pathogen free, 11 weeks old, weighing 210–250 g) were used.

**Organisms**

Organisms were clinical isolates from patients with uterine endometritis. MICs were determined by a standard agar dilution method using Mueller-Hinton agar (Difco Laboratories, Detroit, MI, USA) for Escherichia coli, and Gifu anaerobic medium (GAM) agar (Kyokuto Pharmaceutical Co. Ltd, Tokyo, Japan) for Bacteroides fragilis. The MIC was defined as the lowest concentration of drug that prevented visible growth of bacteria. Aerobic and anaerobic organisms grown on agar plates were suspended in Mueller-Hinton broth (Difco) and GAM broth (Kyokuto) respectively, to obtain about $5 \times 10^8$ cfu/mL. After 200-fold dilution of the suspension, bacteria at a density of about $2.5 \times 10^4$ cfu per spot were inoculated on to agar plates containing each antimicrobial agent with a multipoint inoculator (Microplanter; Sakuma Seisakusho, Tokyo, Japan). A II aerobic cultures were incubated at 37°C for 24 h, and all anaerobic cultures at 37°C for 48 h in a GasPak system (Becton-Dickinson, Cockeysville, MD, USA).

E. coli was grown on Mueller-Hinton agar (Becton-Dickinson) for 24 h, and B. fragilis on Brucella haemin, vitamin K1 rabbit/sheep agar (K yokuto) for 48 h. A aerobic bacterial culture medium (ABCM) broth (Eiken Chemical Co., Ltd., Tokyo, Japan) was used for inoculation into the uterine cavity.

**Rat uterine endometritis model**

Rats were anaesthetized intraperitoneally with sodium pentobarbital at a dosage of 25 mg/kg. The abdominal and flank hair was shaved, and then the abdominal wall was thoroughly swabbed with povidone-iodine. A small
vertical incision was made in the abdominal wall, and uterus and adnexa were exposed. A sterile foreign body, a $1 \text{ mm} \times 7 \text{ mm} \times 1 \text{ mm}$ part of an FD-1 intrauterine device (Fuji Latex Co. Ltd, Tokyo, Japan) was placed into the uterine cavity. Using a disposable sterile tuberculin syringe with a 27-gauge needle, 0.05 mL of bacterial suspension containing $1.1 \times 10^5 \text{ cfu/rat}$ of $E. \text{ coli}$ or $1.3 \times 10^5 \text{ cfu/rat}$ of $B. \text{ fragilis}$ was injected into the right side of uterine cavity. The abdominal wall was closed with 1-0 silk suture. After the operation, the abdominal wound was disinfected with povidone-iodine every day.

Therapeutic study

Sixteen hours after the intrauterine inoculation of $1.1 \times 10^5 \text{ cfu/rat}$ of $E. \text{ coli}$ or $1.3 \times 10^5 \text{ cfu/rat}$ of $B. \text{ fragilis}$, DU-6859a (20 mg/kg po bid) or levofloxacin (20 mg/kg po tid) was given to groups of three rats for 3 days. Control rats received no drug. The dose and frequency of administration had been determined in another study.\textsuperscript{8,9} Eighty-eight hours after bacterial inoculation, under anaesthesia with sodium pentobarbital, the peritoneal cavity was opened aseptically, and the right uterine corpus was resected. Part of it was subjected to histological examination, and most of the uterine corpus was homogenized and subjected to quantitative bacterial examinations. Quantitative bacterial cultures were performed on BTB agar (Eiken Chemical Co. Ltd) for $E. \text{ coli}$ and on bile-aesculin agar (Kyokuto) for $Bacteroides$.

Statistical analysis

Values are reported as mean ± standard deviation (s.d.). All results were analysed using the Bonferroni-Dunn test and significance was assigned at $P < 0.05$.

Results and discussion

The figure shows the histological effects of DU-6859a and levofloxacin on the rat uterine corporis in DU-6859a-treated, levofloxacin-treated and untreated groups. Infections were restricted to the uterus. The inflammatory reactions and leucocyte infiltration of the uterus in the treated groups were milder than those in the untreated group.

The MICs of DU-6859a and levofloxacin for $E. \text{ coli}$ GOG 0020 were 0.025 and 0.05 mg/L, respectively, and MICs of DU-6859a and levofloxacin for $B. \text{ fragilis}$ GOG 3101 were 0.20 and 0.39 mg/L, respectively.

The Table shows the bacteriological effects of DU-6859a and levofloxacin. The viable cell counts of $E. \text{ coli}$ and $B. \text{ fragilis}$ in the DU-6859a- and levofloxacin-treated groups were significantly lower than those in the untreated group.

Animal models can be indispensable for evaluating the therapeutic effects of antimicrobial agents, especially in studies of infectious diseases. Data from animal models often provide information that is valuable for human infectious diseases, dealing with bacterial virulence and

Figure. Histological analysis of the uterine corporis in (a) DU-6859a-treated, (b) levofloxacin-treated and (c) untreated groups of rats.
Therapeutic effects of DU-6859a

Table. Bacteriological effects of DU-6859a and levofloxacin on uterine corporis inoculated with E. coli or B. fragilis (data given as viable cell counts in uterine corporis, in log cfu/g)

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>DU-6859a E. coli</th>
<th>DU-6859a B. fragilis</th>
<th>Levofloxacin E. coli</th>
<th>Levofloxacin B. fragilis</th>
<th>Untreated E. coli</th>
<th>Untreated B. fragilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.56</td>
<td>1.96</td>
<td>3.07</td>
<td>3.02</td>
<td>5.68</td>
<td>6.58</td>
</tr>
<tr>
<td>2</td>
<td>3.05</td>
<td>3.21</td>
<td>2.96</td>
<td>3.45</td>
<td>6.95</td>
<td>6.35</td>
</tr>
<tr>
<td>3</td>
<td>2.88</td>
<td>2.85</td>
<td>3.01</td>
<td>3.14</td>
<td>6.21</td>
<td>7.06</td>
</tr>
<tr>
<td>Mean</td>
<td>2.83a</td>
<td>2.67a</td>
<td>3.01a</td>
<td>3.20a</td>
<td>6.28</td>
<td>6.67</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.25</td>
<td>0.64</td>
<td>0.06</td>
<td>0.22</td>
<td>0.64</td>
<td>0.36</td>
</tr>
</tbody>
</table>

*aCompared with untreated group; P < 0.0001 by Bonferroni-Dunn test.

therapeutic efficacy. There have been few but limited animal models of intrauterine infection. A model of pyometra has been tried, but was unsuccessful.10 Since the pyometra model is a closed-phase one, it is similar to an in-vitro model. We believe that the endometritis model is better than the pyometra model for evaluating drug efficacy as it is closer to the clinical situation. Therefore, we designed a rat model of intrauterine infection in which a foreign body is used to promote persistence of endometrial infection.

These results suggest that DU-6859a, as well as levofloxacin, might be useful for treating polymicrobial infections in the clinical fields of obstetrics and gynaecology.

References


Received 4 November 1996; returned 17 February 1997; revised 25 June 1997; accepted 1 August 1997