

Studies on the Pathogenicity of Anaerobes, Especially *Prevotella bivia*, in a Rat Pyometra Model

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ABSTRACT

Objective: *Prevotella bivia* is one of the anaerobic bacteria that resides in the flora of the female genital tract. We studied the pathogenicity of *P. bivia* in a rat pyometra model.

Methods: The experimental animal (rat) model of pyometra was developed to investigate the pathogenicity of *P. bivia* in a rat pyometra model.

Results: In the groups inoculated with aerobes alone, the infection rate was 10% (1/10) in the *Staphylococcus aureus*- or *Staphylococcus agalactiae*-inoculated group and 20% (2/10) in the *Escherichia coli*-inoculated group. Infection was not established in the groups inoculated with anaerobes alone. High infection rates were observed in all the mixed-infection groups. In the *S. agalactiae*- and *Bacteroides fragilis*-, *S. agalactiae*- and *P. bivia* -, *E. coli*- and *B. fragilis*-, and *E. coli*- and *P. bivia*-inoculated groups, an infection rate of 100% (10/10) was demonstrated. The efficacy of antibiotics such as flomoxef (FMOX) could be determined using a rat pyometra model. In relation to the alteration of vaginal microbial flora during the menstrual cycle, estrogen increased the growth of *P. bivia*.

Conclusion: Mixture of aerobic bacteria and *P. bivia* increased the pathogenicity of *P. bivia*. Estrogen would be useful for raising up the inflammatory change of the uterus in experimental models of genital tract infection due to *P. bivia*. Infect. Dis. Obstet. Gynecol. 6:61-65, 1998.

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KEY WORDS

Prevotella bivia; estrogen; experimental model

We have studied the clinical significance of anaerobic bacteria, especially *Prevotella bivia* in obstetrics and gynecology.¹ There have been no animal model work associated with *P. bivia* in obstetrics and gynecology. An experimental animal (rat) model of pyometra has been used in this study. Using this model, pathogenicity of *P. bivia* was determined in the association of mixed infection with aerobic bacteria. The adequacy of a rat pyometra model was evaluated for the administration of efficacy of antibiotics. Since *P. bivia* is predominantly isolated in the vaginal cavity during the

follicular phase,¹ the growth of *P. bivia* might be associated with sex steroid hormones, especially estrogen and progesterone. Additionally, the effects of estrogen and progesterone on the growth of *P. bivia* were studied using this model.

MATERIALS AND METHODS

Study on the Pathogenicity of *P. bivia* in an Experimental Animal (Rat) Model Bacterial Strains

Clinical isolates such as *Staphylococcus aureus*, *Streptococcus agalactiae*, *Enterococcus faecalis*, *Enterococcus*

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faecium, *Escherichia coli*, *Peptostreptococcus anaerobius*, *Bacteroides fragilis* and *P. bivia* were obtained from pyometra in human subjects and used the following experiments.

Animals

Each group consisted of 10 female Wistar rats, 9 to 13 weeks old, weighing 170 to 250 grams.

Intrauterine Infection (Pyometra) of Rats

Eighteen groups of rats were inoculated with the following bacterial suspensions: (a) *S. aureus*, (b) *S. agalactiae*, (c) *E. faecalis*, (d) *E. faecium*, (e) *E. coli*, (f) *P. anaerobius*, (g) *B. fragilis*, (h) *P. bivia*, (i) *S. aureus* and *P. bivia*, (j) *S. agalactiae* and *P. anaerobius*, (k) *S. agalactiae* and *B. fragilis*, (l) *S. agalactiae* and *P. bivia*, (m) *E. faecalis* and *P. bivia*, (n) *E. coli* and *P. anaerobius*, (o) *E. coli* and *B. fragilis*, (p) *E. coli* and *P. bivia*, (q) Mueller Hinton Broth (Becton Dickinson and Company, Cockeysville, MD), and (r) GAM broth (Nissui Pharmaceutical Co., Ltd., Japan).

For the bacterial suspension used for inoculation, the aerobes grew on Mueller Hinton Agar (Becton Dickinson) for 24 hours and anaerobes on modified GAM agar (Nissui) for 48 hours. The colonies were emulsified in modified GAM broth and the emulsion was adjusted to a concentration of 10^5 colony-forming units (cfu)/rat.

Each rat was anesthetized by intraperitoneal injection of 25 mg of pentobarbital (Nembutal, Dainippon Pharmaceutical Co., Ltd., Japan) and underwent laparotomy in the supine position. The cervical canal of the uterus was closed with the ligation of surgical silk 1-0, avoiding injury of the uterine arteries. Fifty μ L bacterial suspension was injected through a microsyringe into the right uterine horn. The site of injection was sealed by an instant adhesive, Aronalph A (Sankyo Co., Ltd., Japan), to prevent the outflow. Aronalph A did not cause inflammatory change of the uterus. The left uterine horn was used as a control.

Judgement of the Presence of Infection (Pyometra)

The rats were sacrificed 7 days after bacterial inoculation. The uterus was removed, and the retained intrauterine fluid was collected and examined bacteriologically for the quantitative determination. Quantitative assays were performed by the dilution method with GAM broth. Sheep blood

agar (Becton Dickinson) and Brucella HK (hemin, vitamin K₁) RS (rabbit, sheep) blood agar (Kyokuto Pharmaceutical Co., Ltd., Japan) were used as isolation media. Simultaneously, the endometrium was histologically examined for the presence of inflammation. Inflammations were judged by the presence of both the bacteria in the fluid and neutrophil accumulation on histological examination.

Study of the Efficacy of Flomoxef for the Treatment of *P. bivia*-Infected Pyometra

Bacterial Strains

Clinical isolates of *E. coli* and *P. bivia* from pyometra in human subjects were used. MIC values were determined according to the standard method of the Japan Society of Chemotherapy.² The MIC was defined as the lowest drug concentration that was preventable for any visible growth of bacteria.

Animals

Six female Wistar rats, 9 weeks old, weighing 200 to 220 grams, were used.

Experiments

Efficacy of FMOX was determined in rat pyometra infected with *E. coli* or *P. bivia*. On the second day of bacteria inoculation, flomoxef sodium (-(6R, 7R)-7-[2-(difluoromethylthio) acetamido] -7-methoxy-3-[[1-(2-hydroxyethyl)-1H-tetrazol-5-yl] thio-methyl]-8-oxo-5-oxa-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylate), 20 mg/kg twice daily, was injected intravenously to the rats for 3 days. On the day following the final injection, the rats were sacrificed. The viable cell counts (\log_{10} cfu/mL) in the uterine fluid were determined.

Effects of Estrogen and Progesterone on Rat Intrauterine Infection (Pyometra) Caused by *E. coli* and *P. bivia*

Drugs

Estradiol benzoate (Mochida Pharmaceutical Co., Ltd., Japan) and progesterone (Teikoku Zoki Co., Ltd., Japan) were used.

Bacterial Strains

Clinical isolates of *E. coli* and *P. bivia* were obtained from pyometra in human subjects.

TABLE 1. The incidence of pyometra by various bacteria in the experimental model

Organisms used	Infection rate
<i>Staphylococcus aureus</i>	1/10 (10%)
<i>Streptococcus agalactiae</i>	1/10 (10%)
<i>Enterococcus faecalis</i>	0/10 (0%)
<i>Enterococcus faecium</i>	0/10 (0%)
<i>Escherichia coli</i>	2/10 (20%)
<i>Peptostreptococcus anaerobius</i>	0/10 (0%)
<i>Bacteroides fragilis</i>	0/10 (0%)
<i>Prevotella bivia</i>	0/10 (0%)
<i>S. aureus, P. bivia</i>	8/10 (80%)
<i>S. agalactiae, P. anaerobius</i>	5/10 (50%)
<i>S. agalactiae, B. fragilis</i>	10/10 (100%)
<i>S. agalactiae, P. bivia</i>	10/10 (100%)
<i>E. faecalis, P. bivia</i>	6/10 (60%)
<i>E. coli, P. anaerobius</i>	7/10 (70%)
<i>E. coli, B. fragilis</i>	10/10 (100%)
<i>E. coli, P. bivia</i>	10/10 (100%)
Mueller Hinton Broth	0/10 (0%)
GAM broth	0/10 (0%)

Animals

Each group consisted of three female Wistar rats, 9 to 13 weeks of age, weighing 170 to 250 grams.

Experiments

Rats with intrauterine infection (pyometra) were segregated into three groups to study the effects of estrogen and progesterone. Rats were injected intramuscularly with estradiol benzoate in oil (0.2 mg/rat) or with progesterone in oil (2 mg/rat) once a day for 7 days. Injections were started one day before bacterial inoculation. Each rat underwent laparotomy in the supine position. The inoculation of *E. coli* and *P. bivia* was performed as previously described. The rats were sacrificed 7 days after bacterial inoculation, and the viable bacterial count in the uterine fluid was determined as log₁₀ cfu/mL.

RESULTS

Study of the Pathogenicity of *P. bivia* in an Experimental Pyometra Model

In the pyometra model, inflammation was limited in the endometrium and myometrium. The left uterus had no inflammatory signs by injection of bacterial free fluid into the right uterus. These findings indicate adequacy as a pyometra model.

Mixed infections focused on *P. bivia* were studied in a pyometra model. The results are shown in Table 1.

In the groups inoculated with aerobes alone, the

TABLE 2. Therapeutic effects of flomoxef (FMOX) on pyometra in a rat model^a

	Viable cell counts (log ₁₀ cfu/mL)		
	Rat no.	FMOX-treated group ^b	Untreated group
<i>Escherichia coli</i>	1	2.8	5.4
	2	0.0	4.7
	3	2.6	5.0
	Mean ± SD	1.8 ± 1.6*	5.0 ± 0.4
<i>Prevotella bivia</i>	1	2.9	7.0
	2	3.2	8.2
	3	3.2	7.2
	Mean ± SD	3.1 ± 0.2*	7.5 ± 0.6

^acfu, colony-forming units; SD, standard deviation.

^bFlomoxef (20 mg/kg) was intravenously administered twice daily for 3 days 24 hours after inoculation of *Escherichia coli*.

*P < 0.05, significantly different from the untreated group by t-test.

infection rate was 10% (1/10) in the *S. aureus*- and *S. agalactiae*-inoculated groups and 20% (2/10) in the *E. coli*-inoculated group. Infection was not established in the groups inoculated with anaerobes alone.

High infection rates were observed in all the mixed-infection groups: *S. agalactiae* and *B. fragilis*, 100% (10/10); *S. agalactiae* and *P. bivia*, 100% (10/10); *E. coli* and *B. fragilis*, 100% (10/10); and *E. coli* and *P. bivia*, 100% (10/10).

Efficacy of Flomoxef on Intrauterine Infection (Pyometra) by *P. bivia* in a Rat Model

The efficacy of FMOX treatment of pyometra infected by *E. coli* and *P. bivia* in a rat model is shown in Table 2. The MICs of FMOX to *E. coli* and *P. bivia* strains were 0.20 and 1.56 µg/mL, respectively.

In the untreated group, the amount of *P. bivia* and *E. coli* increased markedly in comparison with the FMOX-treated group (P < 0.05 by t-test).

Effects of Estrogen and Progesterone on Pyometra Infected by *E. coli* and *P. bivia* in a Rat Model

The effects of estrogen and progesterone on pyometra infected by *E. coli* and *P. bivia* are shown in Table 3. The amount of *E. coli* and *P. bivia* was significantly higher in the group treated with estrogen than in the groups treated with progesterone and the untreated group (P < 0.05 by t-test).

DISCUSSION

Bartlett et al.³⁻⁷ developed a rat and mouse intraperitoneal abscess model using *E. coli* and *B. fragilis*

TABLE 3. Hormonal effects on bacterial growth in a rat pyometra model^a

	Rat no.	Viable cell counts (log ₁₀ cfu/mL)		
		Estrogen-treated	Progesterone-treated	Untreated
<i>Escherichia coli</i>	1	6.3	5.3	5.0
	2	6.3	5.2	5.0
	3	6.7	5.1	4.9
	Mean ± SD	6.4 ± 0.2*	5.2 ± 0.1	5.0 ± 0.1
<i>Prevotella bivia</i>	1	6.5	5.3	5.2
	2	6.2	5.2	5.1
	3	6.2	5.4	5.3
	Mean ± SD	6.3 ± 0.2*	5.3 ± 0.1	5.2 ± 0.1

^acfu, colony-forming units; SD, standard deviation.

*P < 0.05, significantly different from the progesterone-treated group and the untreated group by t-test.

to study human intraperitoneal infections. Okada⁸ investigated experimental models for genital tract infection due to anaerobic bacteria, especially associated with *B. fragilis*. This is the first report on animal model work associated with *P. bivia* in obstetrics and gynecology.

Using the rat pyometra model, we demonstrated that inflammation was limited in the endometrium and myometrium. In a mixed infection induced by both an aerobe, such as *E. coli* or *S. agalactiae*, and an anaerobe, such as *B. fragilis* or *P. bivia*, the rate of inflammatory change of the uterus was high.

Efficacy of antibiotics could be determined using the pyometra model. Generally, in cases of suspected mixed aerobic and anaerobic infections, the number of anaerobes tends to increase, indicating necessary administration of antimicrobial agents to which anaerobes are susceptible. The present study clearly demonstrated the important role of anaerobes in obstetric and gynecologic infections, which reminds clinicians of infections of anaerobes such as *Peptostreptococcus* spp., *B. fragilis*, *P. bivia*, *P. disiens*, and *F. nucleatum*.

Alteration of vaginal bacterial flora during the menstrual cycle is noted in the limited reports of Morris,⁹ Neary et al.,¹⁰ Corbishley,¹¹ Sparks et al.,¹² and Ohashi.¹³ In our study, *B. fragilis* and *P. bivia* were isolated at high frequency in the luteal phase and in the follicular phase, respectively.¹ Animal experiments in this study demonstrate that the growth of *P. bivia* and *E. coli* is promoted by estrogen but not by progesterone. Those indicate the presence of estrogen effect on the growth of *P. bivia*. Although Okada⁸ used progesterone for raising up the inflammatory change of the uterus, estrogen would be useful for raising up the inflam-

matory change of the uterus in experimental models of genital tract infection due to *P. bivia*.

Osborne et al.¹⁴ reported that there is no difference in the detected rate of bacteria isolated in cervical and uterine cavities before and after menopause. However, the isolation rate of anaerobes to aerobes is high both in premenopausal women and in postmenopausal women who take estrogen.¹⁴ Therefore, the growth of bacteria might be partly regulated by sex steroids.

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