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Transvascular dissemination of *Porphyromonas gingivalis* from a sequestered site is dependent upon activation of the kallikrein/kinin pathway

S.-W. Hu[1], C.-H. Huang[2], H.-C. Huang[2], Y.-Y. Lai[2], Y.-Y. Lin[1]
[1]Department of Dentistry and [2]Institute of Stomatology, College of Oral Medicine, Chung Shan Medical University, Taichung, Taiwan

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Background and Objective: Epidemiological evidence implicates a connection between human periodontitis and systemic diseases. One possible mechanism involves the direct dissemination of periodontopathogens to the target organs through the circulation. The aim of this work was to define the mechanism used by *Porphyromonas gingivalis* for dissemination from a sequestered infection site.

Material and Methods: BALB/c mice were subcutaneously infected with *P. gingivalis* via use of a mouse chamber model. Tissue fluids from various sites were collected and cultured to determine the presence of *P. gingivalis*. Evans Blue dye was used to measure the dissemination ability of *P. gingivalis*. Kinin-associated molecules were introduced into mice, and their effects on bacterial dissemination and mouse pathology were monitored.

Results: *P. gingivalis* strain A7436 caused remote lesions and septicemia with severe cachexia, resulting in animal death. Intrachamber challenge with A7436 resulted in vascular permeability enhancement (VPE), as measured by the systemic infiltration of Evans Blue dye into chamber fluids. VPE was blocked by kininase and kinin receptor antagonist and enhanced by exogenous bradykinin and kininase inhibitor. Live bacteria were recovered from the subcutaneous perichamber and abdominal spaces (spreading), and from the blood (disseminating) of infected mice. Both kininase and kinin receptor antagonist reduced animal mortality as a result of infection with strain A7436 and decreased the number of bacteria recoverable from the blood, but they were not associated with bacterial spreading.

Conclusions: The results suggest that activation of the kinin system is involved in the breach of the vascular barrier that permits dissemination of *P. gingivalis*.

Dr Yuh-Yih Lin, College of Oral Medicine, Chung Shan Medical University, no. 110 Sec. 1 Chien-Kuo N. Road, Taichung, 40201, Taiwan
Tel: +886 4 24718668, ext. 55509
Fax: +886 4 24759065
e-mail: yuhyih@csmu.edu.tw

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Increasing evidence suggests a possible connection between periodontitis and systemic diseases (1–4). The biological rationale for a causal association has been evaluated by Beck *et al.* (5), who indicated several possible pathways of an infectious inflammatory origin of systemic diseases. Mechanisms that periodontal pathogens might use to exert systemic effects include moving from their oral habitat and dissemination to other parts of the host (6).

Porphyromonas gingivalis is a principal etiologic agent of chronic adult periodontal disease (7–9). It is a gram-negative, anaerobic, β-hemolytic, asaccharolytic, black-pigmented short-rod micro-organism. It requires heme and menadione for growth (10). Its purported virulence traits include production of proteinases, fimbriae (11,12), hemagglutinin (13) and hemolysins (14,15), evasion of the protective functions of phagocytic cells (16), and the ability to disseminate and/or invade host tissues (17–20).

There is compelling evidence (21,22) to indicate that kinins are rapidly generated after proteolytic tissue injury by *P. gingivalis* infection, and kinins *per se* seem to modulate many of the events observed during the inflammatory processes, including vasodilatation, increase of vascular permeability, plasma extravasation, and cell migration (23–25).

Studies on *Vibrio vulnificus*, a gram-negative bacterium that causes gastroenteritis and primary septicemia, suggest that its vascular permeability enhancement (VPE) activity caused by the generation of bradykinin (BK) may permit dissemination from its local site of infection (26). The authors concluded that appreciable BK production in the primary infection foci in the mouse peritoneal cavity after bacterial inoculation was critically involved in facilitating the transvascular dissemination of *V. vulnificus*. The current studies were therefore designed to examine potential differences in the abilities of two *P. gingivalis* strains to induce VPE, and to examine the relationships of VPE activities to the disseminating properties of these two strains. In the present experiments, we investigated the role of BK in triggering septicemia caused by *P. gingivalis*. We demonstrate here that blocking the activation of the host kallikrein/kinin system suppresses the VPE response to local infection challenge with *P. gingivalis* and concomitantly abrogates bacterial transvascular dissemination, but does not influence subcutaneous bacterial spreading.

Material and methods

Bacterial strains and growth conditions

Strains A7436 and 381 of *P. gingivalis* were cultured on Brucella blood agar plates, containing hemin and menadione (27), in an atmosphere of 5% CO_2/10% H_2/85% N_2, at 37°C. Bacteria were passaged at least five times, by picking colonies from the plate surface and transferring to fresh blood agar plates, before being used to challenge mice. The bacterial cells were harvested from 24–30-h-old blood agar plate cultures, concentrated by centrifugation at 1000 *g*, and resuspended in Brain-Heart Infusion (BHI) broth.

Bacterial protease activity assay

Bacteria collected from the surface of anaerobically cultured blood agar were washed twice in phosphate-buffered saline (PBS) and resuspended at a concentration of 1×10^{10} colony-forming units (CFU) in 1 ml of Tris buffer (10 mM Tris-HCl, 150 mM NaCl, 1 mM dithiothreitol, pH 7.4), followed by cell lysis by ultrasonication. The solid phase was removed by centrifugation, and the supernatant was used for the protease activity assay. The protease activities of *P. gingivalis* A7436 and 381 were determined using *N*-α-benzoyl-DL-arginine-p-nitroanilide (BAPNA; Sigma Chemical Co., St Louis, MO, USA). Samples were preincubated in 0.5 ml of 0.1 M Tris-HCl, 200 mM Gly-Gly, 5 mM $CaCl_2$, 10 mM cysteine, pH 7.6, for 5 min at 37°C, and then 0.5 ml of 2 mM BAPNA was added. The formation of p-nitroaniline was read spectrophotometrically at 410 nm.

Experimental animals and infection protocol

Each BALB/c female mouse, ≈8 weeks of age, was surgically implanted with a stainless steel coil to create a subcutaneous chamber in the dorsolumbar region (28). After allowing healing to take place for at least 14 days, all mice were infection-challenged by intrachamber injection on day 0 with *P. gingivalis* strain A7436 or strain 381(1×10^9 CFU in 100 μl of BHI) or sham inoculated (with 100 μl BHI) as a control. All experiments were performed in accordance with protocols approved by the Animal Care and Use Committee of Chung Shan Medical University.

Bradykinin (BK), B2 bradykinin receptor antagonist [Kra; D-Arg, (Hyp^3, $Thi^{5,8}$, D-Phe^7)-bradykinin], angiotensin-converting enzyme (ACE, a kininase), and captopril (an ACE inhibitor) were purchased from Sigma Chemical Co. For evaluating the effects of the kinin system on this infection animal model, the above-mentioned kinin-associated molecules were injected intraperitoneally at the time of bacterial challenge and 24 h later. Each mouse was injected with 20 μg of BK, 200 μg of Kra, 500 μg of ACE or 500 μg of captopril in 100 μl of PBS, or with PBS alone as a control.

Fluid samples were taken from chambers, chamber vicinities, mouse abdominal subcutaneous areas, and the retro-orbital venous plexus at 0, 0.3, 1, 3, 6, and 24 h postbacterial infection, as described below.

Quantification of bacteria in various biological fluids

Fluid samples from within the chambers, in the chamber vicinities, and from subcutaneous abdominal areas were aspirated with Hamilton microsyringes (26 gauge) and diluted 1 : 100 in PBS. Blood was taken from the retro-orbital venous plexus using glass capillary tubes coated with heparin. Serial dilutions of fluid samples were streaked onto anaerobic blood agar plates and cultured in an anaerobic atmosphere for at least 7 days. The presence of *P. gingivalis* was confirmed

by the growth of characteristic black colonies and a foul odor emanating from the blood agar plates.

Detection of vascular permeability enhancement

Evans Blue dye (EBD) has been used as a vital dye to investigate vascular injuries that lead to extravasation of blood and plasma proteins (29). EBD was purchased from ICN Biomedicals Inc. (Irvine, CA, USA) and prepared as a 2.5% (w/v) solution in PBS. Mice were injected intraperitoneally with EBD (100 µl) 10 min before bacterial or sham challenge. Chamber fluid (10 µl) was taken from each mouse after bacterial challenge, and then diluted 1 : 10 with PBS in microcentrifuge tubes. Aliquots were centrifuged to remove the solid phase, and placed in a flat-bottom 96-well microtiter plate. VPE activities were determined by spectrophotometry at 610 nm, according to the method of Udaka *et al.* (30).

Table 1. Comparisons of pathology in a mouse chamber model infected with *Porphyromonas gingivalis* strains 381 and A7436

P. gingivalis strain	Local lesion	Remote lesion	Mortality
381 ($n = 18$)	100%[a]	0%	0%
A7436 ($n = 21$)	0%	100%[b]	100%

[a]Local lesions occurred between days 9 and 13 at the challenged sites of infected mice.
[b]Remote lesions occurred between days 1 and 2 on the abdominal areas of infected mice.

Statistical analysis

The results were expressed as the mean ± standard error. The statistical analysis software, JMP (SAS Institute Inc., Cary, NC, USA), was used in this study, and a p-value of < 0.05 was considered significant. Unpaired Student's t-tests were used to examine the differences between experimental and control groups. Log-rank tests were used to analyse animal survival between groups.

Results

Pathology of *P. gingivalis* in a mouse chamber model

Intrachamber challenge with 1×10^9 CFU *P. gingivalis* A7436 resulted in 100% death within 48 h (Table 1). All infected animals showed severe cachexia with piloerection, hunched backs and trembling limbs. There were no lesions or swellings at the sites of bacterial injection. Instead, there were remote lesions in the abdominal areas that appeared between 24 h and 48 h postchallenge with black exudates. Death was consistent with septic shock. In contrast, no animals died following infection challenge with *P. gingivalis* 381. All animals infected with *P. gingivalis* 381 developed perichamber lesions within 14 d of challenge.

Bacteria recovered from various biological fluids

Tissue fluids taken from within the chambers, the chamber vicinities, the abdominal areas, and the retro-orbital postcapillary venules were collected during the course of bacterial infection. Bacteria were persistently recoverable from within the chambers, with statistically significantly higher numbers of *P. gingivalis* 381 recovered between 0.3 and 6 h postbacterial challenge than of *P. gingivalis* A7436 (Table 2). *P. gingivalis* 381 was never detected in fluids other than intrachamber fluid, indicating the inability of this strain to breach the confinement of the chamber (Tables 3, 4 and 5). On the other hand, *P. gingivalis* A7436 was recovered by 20 min postchallenge from the subcutaneous spaces around the chamber. The number of bacteria recoverable from extrachamber spaces increased with time until 6 h postinfection challenge, and the bacteria successfully breached the confinement of chambers in all infected animals (Table 3). Furthermore, *P. gingivalis* A7436 first appeared within 1 h in fluids collected from the subcutaneous spaces associated with the abdomen, and the number of bacteria increased to 1.5×10^6 CFU/ml before perforation of the abdominal skin at 24 h (Table 4). *P. gingivalis* A7436 emerged in the blood 6 h postchallenge (Table 5). The number of bacteria recoverable from blood was 162 ± 65 CFU/ml. After 24 h of infection, the number of bacteria in the blood reached 251 ± 109 CFU/ml. It is worthy of note that *P. gingivalis* was recovered from all extrachamber sample fluids (perichamber fluids, peri-abdominal fluids, and retro-orbital blood), from all animals infected with *P. gingivalis* A7436, by 24 h.

Table 2. Porphyromonas gingivalis recovered from infected foci (inside chambers)

		Time-point postbacterial challenge					
P. gingivalis strain		0 h	0.3 h*	1 h*	3 h*	6 h*	24 h
381	Number of mice[a]	0/7	7/7	7/7	7/7	7/7	7/7
	Recoverable bacteria[b]	0	8.95×10^7 (1.45×10^7)	3.22×10^6 (0.79×10^6)	3.29×10^3 (1.00×10^3)	3.32×10^3 (0.85×10^3)	8.49×10^3 (3.32×10^3)
A7436	Number of mice[a]	0/7	7/7	7/7	7/7	7/7	7/7
	Recoverable bacteria[b]	0	2.93×10^7 (0.87×10^7)	1.94×10^4 (0.65×10^4)	4.47×10^2 (1.02×10^2)	9.55×10^2 (3.35×10^2)	2.20×10^3 (0.66×10^3)

*Significant differences between *P. gingivalis* 381 and A7436 recoverable bacteria at 0.3, 1, 3, and 6 h postbacterial challenge (Student's t-test).
[a]Number of mice from which *P. gingivalis* was recoverable/total number of mice tested.
[b]Mean colony-forming units (standard error) of recoverable *P. gingivalis* per ml of chamber fluid.

Table 3. Porphyromonas gingivalis recovered from subcutaneous perichamber tissues (outside chambers)

P. gingivalis strain		Time-point postbacterial challenge					
		0 h	0.3 h*	1 h*	3 h*	6 h*	24 h*
381	Number of mice[a]	0/7	0/7	0/7	0/7	0/7	0/7
	Recoverable bacteria[b]	0	0	0	0	0	0
A7436	Number of mice[a]	0/7	2/7	3/7	4/7	7/7	7/7
	Recoverable bacteria[b] ($\times 10^5$)	0	0.13 (0.08)	0.17 (0.11)	0.81 (0.11)	2.10 (0.73)	1.16 (0.72)

There were significant differences between *P. gingivalis* 381 and A7436 recoverable bacteria at 0.3, 1, 3, 6, and 24 h postbacterial challenge (Student's *t*-test).
[a]Number of mice from which *P. gingivalis* was recoverable/total number of mice tested.
[b]Mean colony-forming units of recoverable *P. gingivalis* (standard error) from positive sites.

Table 4. Porphyromonas gingivalis recovered from subcutaneous abdominal sites of infected mice

P. gingivalis strain		Time-point postbacterial challenge					
		0 h	0.3 h	1 h*	3 h*	6 h*	24 h*
381	Number of mice[a]	0/7	0/7	0/7	0/7	0/7	0/7
	Recoverable bacteria[b]	0	0	0	0	0	0
A7436	Number of mice[a]	0/7	0/7	3/7	4/7	7/7	7/7
	Recoverable bacteria[b] ($\times 10^5$)	0	0	0.20 (0.02)	1.70 (0.70)	2.31 (0.83)	15.22 (8.60)

*Significant differences between *P. gingivalis* 381 and A7436 recoverable bacteria at 1, 3, 6, and 24 h postbacterial challenge (Student's *t*-test).
[a]Number of mice from which *P. gingivalis* was recoverable/total number of mice tested.
[b]Mean colony-forming units of recoverable *P. gingivalis* (standard error) from culture-positive sites.

Table 5. Porphyromonas gingivalis recovered from blood of infected mice

P. gingivalis strain		Time-point postbacterial challenge					
		0 h	0.3 h	1 h	3 h	6 h*	24 h*
381	Number of mice[a]	0/7	0/7	0/7	0/7	0/7	0/7
	Recoverable bacteria[b]	0	0	0	0	0	0
A7436	Number of mice[a]	0/7	0/7	0/7	0/7	4/7	6/7
	Recoverable bacteria[b]	0	0	0	0	162 (65)	251 (109)

*Significant differences between *P. gingivalis* 381 and A7436 recoverable bacteria at 6 and 24 h postbacterial challenge (Student's *t*-test).
[a]Number of mice from which *P. gingivalis* was recoverable/total number of mice tested.
[b]Mean colony-forming units (standard error) of recoverable *P. gingivalis* per ml of blood from culture-positive animals.

Protease activities of *P. gingivalis* A7436 and 381

Whole cell extracts of *P. gingivalis* A7436 and 381 were assayed for their protease activities on BAPNA. Bacteria were collected in the same way as for mouse challenge, and the same bacterial concentration (1×10^{10} CFU in 1 ml of solution) was used for enzyme assay. *P. gingivalis* A7436 showed significantly higher activities than *P. gingivalis* 381, the respective absorbances at 410 nm being 0.5555 ± 0.0315 vs. 0.0978 ± 0.0035 (Fig. 1).

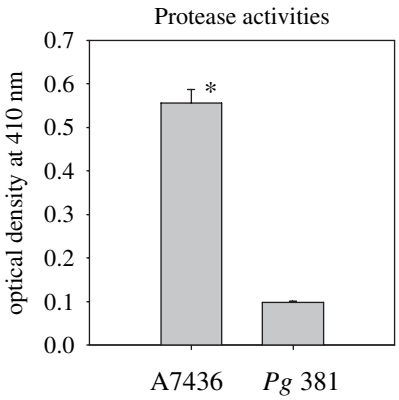

Fig. 1. Protease activities of *Porphyromonas gingivalis* A7436 and 381 using *N*-α-benzoyl-DL-arginine-p-nitroanilide (BAPNA) as the substrate. The same concentrations of bacteria (1×10^{10} colony-forming units/ml) were used in this *in vitro* assay as in animal experiments. The results are expressed as the mean ± standard error. *Strain A7436 showed significantly higher enzyme activities than *P. gingivalis* 381 ($p < 0.001$, Student's *t*-test; $n = 5$ for both strains).

Effects of *P. gingivalis* infection on the vascular permeability of chambers

Systemically administered EBD does not have access to the sequestered environment created by the chamber as there was no detectable infiltration of EBD into chamber fluids that were unchallenged or sham-challenged with BHI (Fig. 2). EBD concentrations in infected chamber fluids were used as a quantitative measure of the different VPE responses to the two *P. gingivalis* strains. There was a rapid increase of VPE activity in response to *P. gingivalis* A7436 infection challenge, as the absorbances at 610 nm reached a plateau of 0.27 within 40 min following infection challenge. In contrast, there was no discernible change in absorbance at 610 nm in response to intrachamber challenge with *P. gingivalis* 381 through the 2 h of monitoring (Fig. 2).

Effects of exogenous BK, Kra, ACE, and captopril on the VPE of chambers and on bacterial recovery following *P. gingivalis* A7436 infection

Previous studies have shown that gingipains from *P. gingivalis* were capable

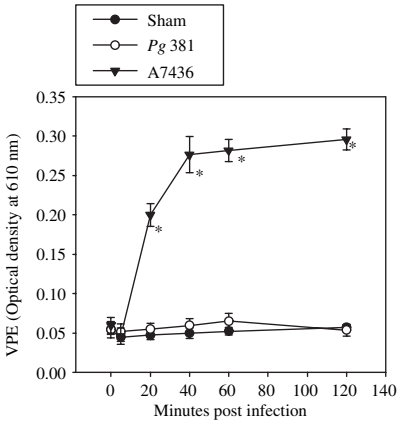

Fig. 2. Concentration of Evans Blue dye in chambers infected with *Porphyromonas gingivalis*. The results are expressed as the mean ± standard error. *Significant differences between *P. gingivalis* A7436 and sham infection in chamber dye concentrations at 20, 40, 60, and 120 min postinfection (Student's *t*-test; $n = 6$ for each group). VPE, vascular permeability enhancement.

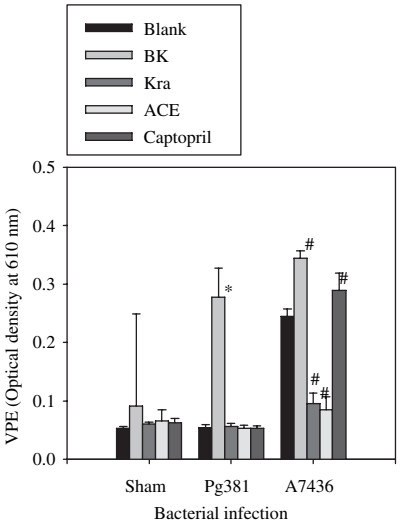

Fig. 3. Effects of treatment with kinin-associated molecules on the concentration of Evans Blue dye in *Porphyromonas gingivalis*-infected chambers. The results are expressed as the mean ± standard error. *Significant difference between bradykinin (BK) and sham treatment groups in chamber dye concentrations at 40 min postinfection with *P. gingivalis* 381 (Student's *t*-test; $n = 7$ for each group). †Significant differences in BK, Kra, angiotensin-converting enzyme (ACE), and captopril treatment groups, when compared with sham treatment groups, in chamber dye concentrations at 40 min postinfection with *P. gingivalis* A7436 (Student's *t*-test; $n = 7$ for each group). VPE, vascular permeability enhancement.

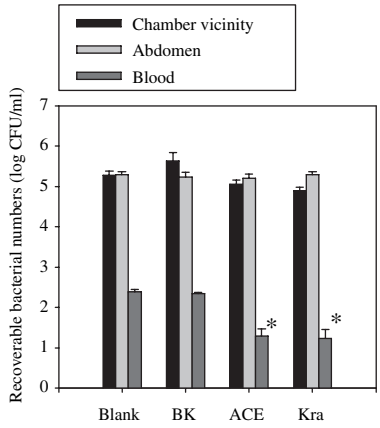

Fig. 4. Site-specific effects of pretreatment with kinin-associated molecules on the numbers of recoverable *Porphyromonas gingivalis* A7436. The results are expressed as the mean ± standard error. *Significant differences in Kra and angiotensin-converting enzyme (ACE) treatment groups, when compared with the blank treatment control, in recoverable bacterial numbers from blood samples at 6 h postinfection with *P. gingivalis* A7436 (Student's *t*-test; $n = 6$ for each group).

of activating the kinin/kallikrein pathway, leading to the production of bioactive BK (31). We inhibited the effects of BK in this animal model by the intraperitoneal administration of 200 μg of Kra just prior to bacterial challenge and 24 h later. The vascular permeability of chambers (Fig. 3) infected with *P. gingivalis* A7436 was lowered in Kra-treated mice, and consistently fewer bacteria were recovered from their blood than from sham-treated mice (Fig. 4). Interestingly, fluids from the perichamber and peri-abdominal spaces did not show significant differences between these two groups (Fig. 4). Treatment with ACE also reduced the concentration of EBD in the chambers of *P. gingivalis* A7436-infected mice (Fig. 3), and resulted in even lower numbers of recoverable bacteria than treatment with Kra (Fig. 4). When animals were treated with exogenous BK or captopril, a significant augmentation in the vascular permeability of chambers was seen (Fig. 3) in *P. gingivalis* A7436-infected mice compared with that of sham-treated animals (0.345 ± 0.013 and 0.289 ± 0.022, respectively, vs. 0.245 ± 0.012 in control mice, at an absorbance of 610 nm).

Effects of exogenous BK, Kra, ACE, and captopril on the vascular permeability of chambers and bacterial recovery following *P. gingivalis* 381 infection

When *P. gingivalis* 381-infected animals were treated with exogenous BK, there was a fourfold elevation in vascular permeability compared with sham-treated animals (Fig. 3). Treatment with Kra, ACE, or captopril had no effect on the concentration of EBD in the chamber when mice were infected with *P. gingivalis* 381. Two out of six exogenous BK-pretreated mice were found to be positive for *P. gingivalis* 381 (Table 6) ($6.50 \pm 1.00 \times 10^2$ CFU/ml), in blood cultures, 6 h after infection challenge. Bacteria were not found in other fluid samples (from perichamber or peri-abdominal spaces), except in chambers (Table 6), and the number of bacteria inside the chambers ranged from 3.02 to 8.84×10^3 CFU/ml in all fluid samples from pretreated animals infection-challenged with *P. gingivalis* 381.

Effects of exogenous BK, Kra, and ACE on the survival rates of animals infected with *P. gingivalis*

Normal mice challenged with 1×10^9 CFU of *P. gingivalis* A7436 in the chambers showed a 100% mortality rate within 2 d. Pretreatment of mice with exogenous BK or captopril did not change the mortality outcomes (Fig. 5). When animals were pretreated with Kra or ACE, there were significant reductions in mortality rates resulting from *P. gingivalis* A7436 infection compared with that of sham-pretreated mice (Fig. 5). There were 17% and 33% animals surviving *P. gingivalis* A7436 infection in Kra and ACE groups, respectively. Longer survival times and milder cachexia were observed in both groups than in the sham-treatment group.

Table 6. The effects of pretreatment with kinin-associated molecules on the numbers of *Porphyromonas gingivalis* 381 recovered from infected mice

		Tissue fluids			
		Inside chambers	Chamber vicinities	Abdomen	Blood
Blank	Number of mice[a]	6/6	0/6	0/6	0/6
	Recoverable bacteria[b]	5.62×10^3 (1.20×10^3)	0	0	0
BK	Number of mice[a]	6/6	0/6	0/6	2/6
	Recoverable bacteria[b]	8.84×10^3 (2.16×10^3)	0	0	6.50×10^2 (1.00×10^2)*
Kra	Number of mice[a]	6/6	0/6	0/6	0/6
	Recoverable bacteria[b]	4.75×10^3 (2.82×10^3)	0	0	0
ACE	Number of mice[a]	6/6	0/6	0/6	0/6
	Recoverable bacteria[b]	3.02×10^3 (8.24×10^2)	0	0	0

*Significant difference in the number of bacteria recoverable between bradykinin (BK) and blank-pretreated mice at 6 h postbacterial challenge (Student's *t*-test).
[a]Number of mice from which *P. gingivalis* was recoverable/total number of mice tested.
[b]Mean colony-forming units (standard error) of recoverable *P. gingivalis* per ml of blood from culture-positive animals.
ACE, angiotensin-converting enzyme.

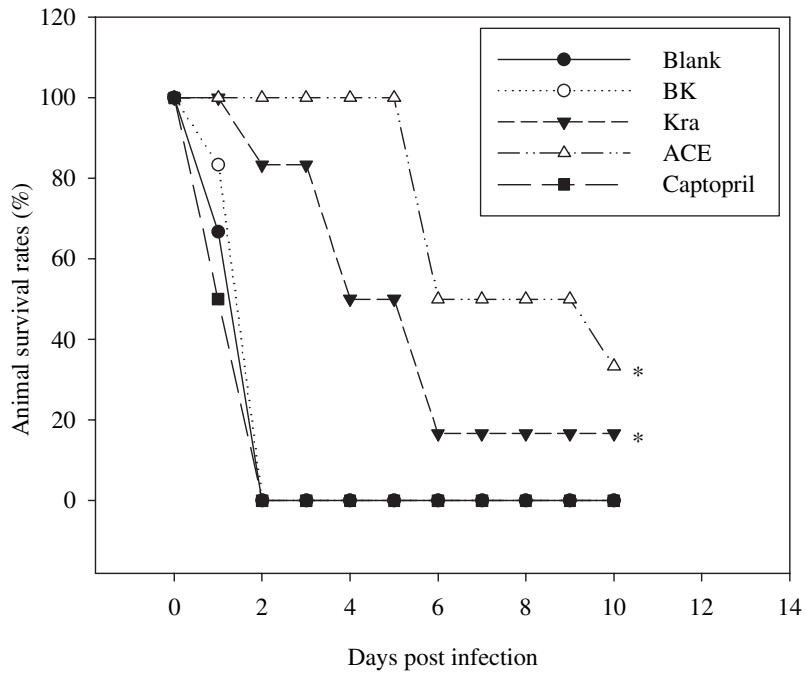

Fig. 5. Survival curves of *Porphyromonas gingivalis* A7436-infected animals treated with kinin-associated molecules. *Significant differences in Kra and angiotensin-converting enzyme (ACE) treatment groups were found, compared with the blank treatment control, in animal survival rates during 10 d postinfection with *P. gingivalis* (survival analysis, log-rank tests; $n = 6$ for each group). BK, bradykinin.

Pretreatment of mice with exogenous BK, captopril, Kra, or ACE in *P. gingivalis* 381 infection challenges did not result in any discernible systemic reactions, even though two out of six mice pretreated with BK were found to have bacteria in their circulation. No deaths occurred in these infected mice, and all perichamber lesions were developed by day 15 (data not shown).

Discussion

There are two strains of *P. gingivalis* defined by their pathologies presented in this animal model. *P. gingivalis* 381 results in local infection and local tissue destruction without manifestation of systemic influences. On the other hand, intrachamber infection challenge with *P. gingivalis* A7436 results in remote lesions in the abdominal areas, progressing to animal mortality outcome. The differences in the resulting pathologies in this animal model may be attributed to their distinct abilities to disseminate. In this study, we clearly demonstrated that *P. gingivalis* A7436 was present in the subcutaneous tissue fluids (chamber vicinity and abdomen) and the blood, through recovery on anaerobic agar plates, while animals challenged with *P. gingivalis* 381 were negative for dissemination and spreading. *P. gingivalis* A7436 was found outside the inoculation sites as early as 20 min postchallenge and entered the bloodstream 6 h postchallenge.

In an effort to characterize the bacterial dissemination of *P. gingivalis* A7436, we found that local vascular permeability was significantly elevated in the infected foci compared with those in sham and *P. gingivalis* 381 infections. Travis *et al.* (21,25,32,33) indicated an involvement of the activated kallikrein/kinin pathway with the induction of vascular permeability and *P. gingivalis* dissemination. To further elucidate the relationship between the kinin system and *P. gingivalis* pathology, exogenous BK and other associated molecules were introduced into the animal model. Both vascular permeability and intravascular bacterial recovery assays showed a positive association with the activated kinin system, and inhibition of the kinin system repressed vascular permeability and bacterial recovery in *P. gingivalis* A7436 infection. Interestingly, the bacterial numbers of *P. gingivalis* A7436 recovered from extra-chamber subcutaneous fluids (chamber vicinities and abdominal areas) were not affected

by the introduction of kinin-associated proteins, indicating that bacterial extrachamber subcutaneous spreading and transvascular dissemination represent two different virulence properties of *P. gingivalis* A7436 and may involve different effector mechanisms.

The VPE data suggested that activated kinin was generated in the *P. gingivalis* A7436-infected animals, but not in the *P. gingivalis* 381-infected animals. Furthermore, exogenous BK induced VPE in the *P. gingivalis* 381 infection model, supporting the inability of strain 381 to activate the kinin system. The VPE activity of *P. gingivalis* has been proposed to relate with their proteinases (31,34). Imamura and coworkers (35) have shown that VPE activity generated by *P. gingivalis* proteases is dose-dependent and can be inhibited up to 90% by antipain, a specific inhibitor of the Arg-specific cysteine proteinases from *P. gingivalis*. They also demonstrated the induction of VPE activity through the production of plasma kallikrein, and subsequent BK release by *P. gingivalis* proteinases. Therefore, the difference in the *trans*-vascular dissemination of *P. gingivalis* A7436 and 381 may be attributed to the difference of their proteinases. We demonstrated here that *P. gingivalis* A7436 possesses more potent proteinases than strain 381, as measured by BAPNA cleavage. Whether these proteinases are responsible for the activation of the kallikrein/kinin system in this animal study warrants further investigation. The other possibility is that kinins are being activated as part of the normal inflammatory response, and A7436 infection results in more prominent inflammation in hosts.

BK is a principal inflammatory mediator. It causes one of the most fundamental inflammatory reactions (fluid accumulation and edema formation resulting from enhanced vascular permeability) (36–38). Thus, increased intravascular dissemination of *P. gingivalis* may be explained by the potent action of BK in causing the intercellular junctions of endothelium, at postcapillary venules, to open, allowing the bacteria to invade the circulatory system (26,39).

Abolishment of kinin effects by kininase or kinin receptor antagonist reduced the vascular permeability of infected loci, and eliminated the presence of *P. gingivalis* A7436 in the bloodstream, but had no effects on their subcutaneous spread from chamber to abdominal areas. Animal mortality by *P. gingivalis* A7436 infection is clearly associated with the occurrence of bacteremia and is affected by kinin-associated proteins, suggesting a possible intervention of systemic involvement of *P. gingivalis* infection by manipulating the host kinin/kallikrein system.

The present study provides evidence for a strain-specific ability of *P. gingivalis* to disseminate to remote sites through activation of the vasoactive kinin system. Such ability might be expected to be a virulence property of the clinical isolates associated with systemic manifestations, including cardiovascular pathologies and abnormal pregnancy outcomes. The possession of such traits should be considered in studies examining the association of periodontopathogens with systemic diseases.

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