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Canine Prostatic Secretions Kill *Trichomonas vaginalis*

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The zinc content of prostatic secretions is thought to be an important nonspecific defense against urinary tract infection in men. This investigation measured killing by prostatic fluid of *Trichomonas vaginalis*, a common sexually transmitted pathogen, and related this activity to zinc concentration. We used a canine model which closely resembles the human male genital tract. Prostatic secretions from all dogs killed all *T. vaginalis* isolates. There appear to be several mechanisms for killing of trichomonads by prostatic fluid. At prostatic fluid zinc concentrations comparable to those in normal men (≥3.2 mM), the rate of killing of trichomonads was proportional to the zinc concentration. At intermediate zinc levels, killing occurred by both zinc-dependent and zinc-independent mechanisms. A zinc-independent mechanism was responsible for antitrichomonal activity at relatively low zinc levels (<1.6 mM), comparable to those in the prostatic fluid of men with chronic prostatitis. This study suggests that the variable clinical spectrum of trichomoniasis in men may result from a balance between the zinc sensitivity of the *T. vaginalis* strains on one side and the content of both zinc and zinc-independent factors in prostatic fluid on the other.

Prostatic secretions are primary components of the antimicrobial defenses of the male genitourinary tract (4, 24, 27). Prostatic fluid antibacterial activity in humans, dogs, and rats is proportional to its zinc concentration (4–6, 13). The antimicrobial spectrum of zinc includes bacteria, viruses, chlamydiae, and fungi (1, 4, 7–9, 17, 23), and zinc present in prostatic fluid may be an important defense against *Trichomonas vaginalis* as well (J. N. Krieger and M. F. Rein, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 21st, Chicago, Ill., abstr. no. 75, 1981). Zinc salts at concentrations found in normal human prostatic fluid (4.5 to 7 mM) kill *T. vaginalis* in vitro and may limit or resolve trichomonal infection in many men. Trichomonads are recovered from the urethra of 70% of men who have contact with infected women within the preceding 48 h, but the prevalence drops to 30% if the interval from contact is 2 weeks (25). It is possible that intermittent bathing of the urethra with zinc-containing prostatic secretions eradicates the protozoa.

Zinc concentrations similar to those in men with chronic prostatitis (<1.6 mM), however, are not trichomonicidal. In addition, clinical *T. vaginalis* isolates vary in zinc sensitivity, and zinc-resistant trichomonads may be selected in vitro. Thus, variation in both the zinc content of host prostatic secretions and the zinc sensitivity of infecting *T. vaginalis* strains may influence the natural history of genitourinary trichomoniasis in men.

Prostatic fluid is a complex biological secretion (10, 20, 22). It is therefore difficult to accept studies of one component of prostatic fluid in artificial medium as the definitive explanation for in vivo events. The purpose of this investigation was to measure the trichomonicidal activity of prostatic fluid and to determine whether this activity was due entirely to its zinc content.

MATERIALS AND METHODS

Canine model. Male mongrel dogs weighing 35 to 40 kg were anesthetized with sodium pentathol, and normal saline was infused intravenously. The surgical procedure was a modification of the method of Huggins (10). The abdomen was doubly prepared with povadone-iodine solution, and surgery was carried out through a low midline transperitoneal incision. The ureters were divided at the level of the bladder and brought to the skin as bilateral cutaneous ureterostomies. The vasa were doubly ligated and divided, a simple cystectomy was done, and the abdomen was closed. The animal was circumcised and then turned to facilitate sample collection. Prostatic secretions were collected in sterile, acid-cleaned glassware for 1 h after intravenous injection of 25 mg of pilocarpine in physiological saline. Specimens were divided into 5-ml samples and stored at −70°C.

Zinc determination. Zinc levels in prostatic fluid specimens were determined by American Medical Laboratories, Inc., Fairfax, Va., with a Perkin-Elmer atomic absorption spectrometer (no. 305B).

Trichomonads. *T. vaginalis* isolates from women attending the Albemarle County Venereal Disease Clinic Charlottesville, Va., were maintained in modified Feinberg-Whittington medium at 37°C under anaerobic conditions (19) and subcultured every 3rd or 4th day. The zinc sensitivity of the three *T. vaginalis* isolates used in the present study was determined in Feinberg-Whittington medium by the technique described below.

Survival studies. The survival of trichomonads in saline and in prostatic fluid was determined by a time-kill method (J. N. Krieger and M. F. Rein, J. Infect. Dis., in press). Tube cultures of *T. vaginalis* were centrifuged for 10 min at 250 × *g*. The organisms were washed three times in normal saline and counted with a hemacytometer. Tubes containing 5 ml of prostatic fluid or saline were inoculated with approximately 2.5 × 10^3 organisms per ml. At 0, 1, 2, and 4 h, the tubes were blended in a Vortex mixer, 0.1-ml samples were removed, and Falcon polystyrene petri plates (60 by 15 mm; Becton Dickinson Labware, Cockeysville, Md.) were inoculated in triplicate. Diamond medium containing 0.5% purified agar was then added to each plate (11). All manipulations were carried out in an anaerobic workbench (no. 77A; Germfree Laboratories, Inc., Miami, Fla.) at 37°C; the pour plates were incubated anaerobically for 5 days at 37°C, and the resulting colonies were counted with a dissecting microscope.

Analysis of kinetic studies. The killing of *T. vaginalis* by inorganic zinc appears to follow first-order kinetics. For a given zinc concentration, mean percent survival was plotted semilogarithmically against time, and the regression line was calculated. The goodness of fit of the regression lines was evaluated with the *t*-statistic. The slope of the linear regression line for each set of datum points measures the rate of killing of trichomonads. To analyze the effect of different ion concentrations, the slope of each regression line, a measure of the rate of killing, was plotted against zinc concentration. Confidence limits for the slope were calculated for a comparison of specimens (2).

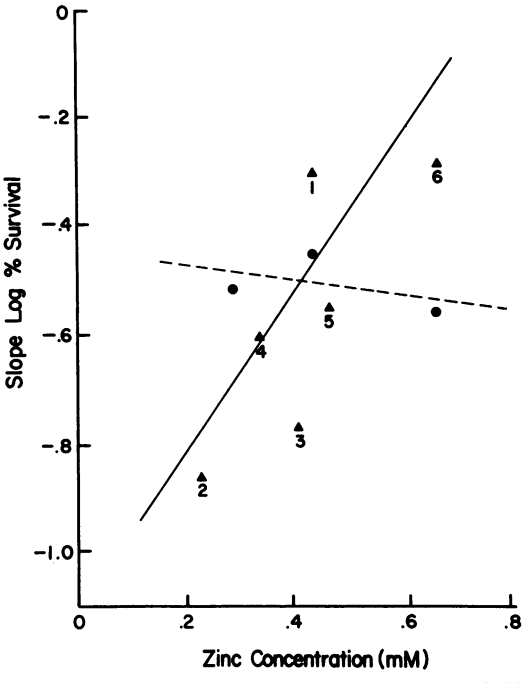

FIG. 1. Survival of *T. vaginalis* in prostatic fluid compared with survival in physiological saline with a similar zinc content. Slope log percent survival, a measure of the rate of killing of trichomonads in saline (●) compared with prostatic secretions (▲) from dogs 1 to 6. Each datum point represents a mean of 9 to 12 determinations. The regression line for trichomonad survival in saline (---) was significantly different from the regression line for survival in prostatic secretions (——) ($P < 0.02$).

RESULTS

The zinc concentrations of the six prostatic fluid specimens ranged from 0.23 to 0.67 mM. The minimal trichomonicidal concentration (MTC) of zinc in saline was 1.6 mM for the three *T. vaginalis* isolates.

Survival of *T. vaginalis* in prostatic fluid from the six dogs was compared with survival in saline containing similar zinc concentrations (Fig. 1). *T. vaginalis* survival in canine prostatic fluid (CPF) was significantly different from survival in saline with a similar zinc content ($P < 0.02$). Survival of trichomonads in saline did not vary significantly over the range of zinc concentrations found in native prostatic fluid. Survival in prostatic fluids, however, was highly variable and appeared to be inversely related to the CPF zinc concentration. *T. vaginalis* survival in some CPF (dogs 1 and 6) was actually greater than survival in saline with a comparable zinc concentration.

Zinc chloride was added to CPF specimens to raise the zinc concentrations to levels which were still below the MTC (Fig. 2). Increasing zinc concentrations to levels below the MTC did not significantly change the rate of killing of trichomonads by prostatic fluid. This supports the finding that at sub-MTC zinc concentrations, killing of trichomonads is independent of the zinc concentration. Furthermore, this suggests that the apparent negative correlation between *T. vaginalis* killing and zinc content of native prostatic fluid was fortuitous, not causal.

The effect of adding $ZnCl_2$ to CPF to achieve a total zinc concentration of 1.6 mM (MTC) is shown in Fig. 3. Trichomonad survival in specimens from three dogs (1, 5, and 6) was not significantly different from survival in normal saline containing 1.6 mM $ZnCl_2$. However, survival of *T. vaginalis* in the other fluids was significantly lower than survival in saline containing 1.6 mM $ZnCl_2$ ($P < 0.05$) (2).

The native prostatic fluids (dogs 2, 3, and 4) which killed trichomonads most rapidly also showed the greatest trichomonicidal activity when their zinc concentration was adjusted to

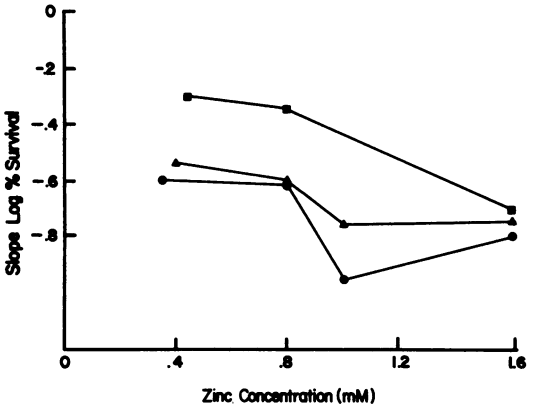

FIG. 2. Survival of *T. vaginalis* in prostatic secretions with subtrichomonicidal zinc levels. Inorganic zinc added to prostatic secretions from dogs 1 (■), 3 (▲), and 4 (●) did not significantly change the rate of killing ($P < 0.05$). Each datum point represents a mean of 9 to 12 determinations.

1.6 mM. It appears that these prostatic fluids contain trichomonicidal factors independent of and additive to the effect of zinc. Adding $ZnCl_2$ to CPF to achieve zinc concentrations above the MTC resulted in *T. vaginalis* survival that was not significantly different from survival in saline with a similar zinc content.

DISCUSSION

Obtaining expressed human prostatic secretions from sexually active men can be difficult and yields microliter amounts. For this reason, many investigators have used ejaculate specimens which yield milliliter sample volumes. Human ejaculate, however, is a composite of secretions: 46 to 80% from seminal vesicles, 13 to 32% from the prostate, and approximately 10% from vasa and epididymides (12, 22). There is also a small contribution from the bulbourethral (Cowper's) glands. Essentially all antibacterial activity has been shown to reside in the prostatic fraction (5, 24). An additional problem with studies in humans is the connection between the genital and urinary tracts, which introduces other variables, e.g., mechanical effects of micturition.

The canine model is particularly suited to studies of the antimicrobial defenses of the lower urinary tract of human males and offers theoretical and practical advantages over clinical studies in humans. Dogs have neither bulbourethral glands nor seminal vesicles (3, 10, 20, 26); thus, bilateral vasectomy, cystectomy, and urinary diversion result in a preparation that yields essentially pure prostatic secretions. Furthermore, pilocarpine stimulation results in 50 to 250 ml of prostatic secretions per dog (10).

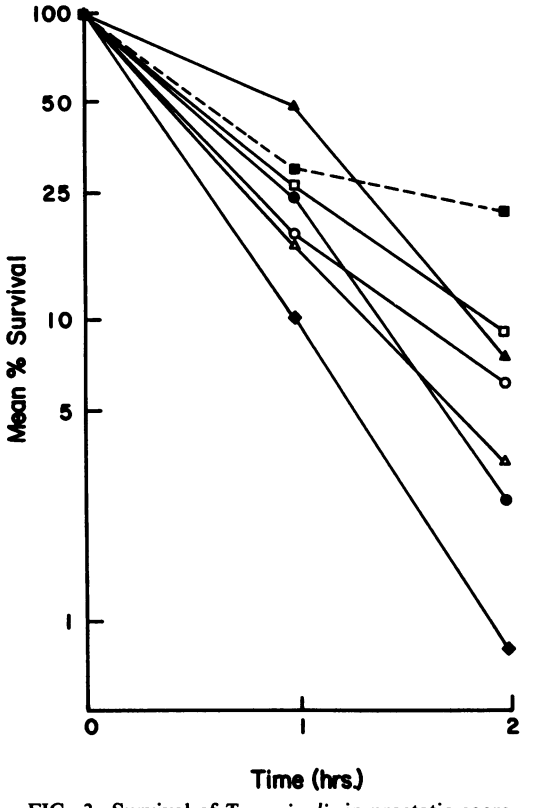

FIG. 3. Survival of *T. vaginalis* in prostatic secretions with zinc levels adjusted to the minimal trichomonicidal concentration (1.6 mM). Survival of trichomonads in prostatic secretions from dogs 1 (□), 5 (▲), and 6 (○) was not significantly different from survival in saline (■) with similar zinc levels. Trichomonad survival in secretions from three dogs was significantly less than survival in saline: 2 (◆; $P < 0.01$), 3 (●; $P < 0.02$), and 4 (△; $P < 0.05$). Each datum point represents a mean of 12 to 15 determinations.

Canine and human prostates (4, 24) are similar in embryology, lack of distinct anatomic and functional lobulation, tendency to undergo spontaneous benign hyperplasia with aging, biochemical composition, and physiological functions.

The major prostatic antibacterial factor in both humans and dogs is a zinc compound (4–6, 13). CPF and human prostatic fluid differ in their zinc content, with the zinc concentration of CPF approximately equal to that found in men with chronic bacterial prostatitis and well below that of normal human prostatic fluid (21) (Table 1). Thus, CPF is particularly useful in searching for zinc-independent antitrichomonal factors.

When zinc was added to CPF so that the total zinc concentration approximated that in normal human prostatic fluid, killing of trichomonads was the same as killing by saline with equal zinc concentrations. It therefore appears likely that

TABLE 1. Zinc concentration in prostatic secretions

Study	Population	No.	Specimen[a]	Mean zinc concn (mM)
Lindholmer and Eliasson (14)	Volunteers	8	SE	4.5
Fair et al. (4)	Genitourology clinic, uninfected chronic bacterial prostatitis	49	EPS	6.9
		15	EPS	0.8
Paz et al. (18)	Healthy, infertility evaluation	53	EPS	4.6
Marmar et al. (15, 16)	Healthy, infertility evaluation	33	EPS	7.0
	Medical students	18	WS	2.3
	Postvasectomy	132	WS	2.3
	Abacterial prostatitis	19	WS	1.4

[a] SE, Seminal plasma in first portion of split ejaculate; EPS, expressed prostatic secretions; WS, whole semen.

in normal men zinc accounts for the trichomonicidal activity of prostatic fluid.

At zinc concentrations similar to those in men with chronic prostatitis (4, 15, 16), some CPF specimens were found to kill trichomonads more rapidly than did the same concentrations of zinc in saline. The independence of trichomonicidal activity and zinc concentrations at such low prostatic fluid levels suggests the presence of an antitrichomonal factor(s) in prostatic fluid in addition to zinc. Some men with sub-MTC levels of zinc may have other host defenses against *T. vaginalis* infection. Thus, the development of trichomonal prostatitis may require a concomitant deficiency of both zinc and zinc-independent factors.

The effectiveness of zinc-independent trichomonicidal factors varies. At zinc concentrations equal to the MTC, prostatic secretions from three dogs killed trichomonads at a significantly faster rate than did saline with a comparable zinc content. These prostatic fluids had the highest native levels of trichomonicidal activity.

We conclude that canine prostatic secretions kill *T. vaginalis* and speculate that this trichomonicidal activity may contribute to the self-limiting nature of trichomonal infection in many men. There appear to be different mechanisms for the killing of trichomonads by prostatic secretions. A zinc-independent mechanism is responsible for antitrichomonal activity at relatively low zinc levels. Such concentrations occur in canines and in men with chronic prostatitis or relative prostatic zinc deficiency. At prostatic fluid concentrations found in normal men, higher than the MTC for most strains of *T. vaginalis*, the rate of killing is proportional to the zinc concentration. At lower zinc concentrations or if the infecting strain is relatively zinc resistant, the killing of trichomonads might occur by both zinc-dependent and zinc-independent mechanisms.

Although they appear to be similar in most respects, human and canine prostatic secretions differ in pH (3) as well as in zinc content. Thus, the findings in the canine or any other animal model should be applied cautiously to the interpretation of human pathophysiology. Further studies with human urogenital secretions are necessary to evaluate the significance of both zinc and zinc-independent mechanisms limiting *T. vaginalis* infection in the lower urinary tract of human males.

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