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Secretion of Various Antimicrobial Substances in Dogs with Experimental Bacterial Prostatitis

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Summary. Bacterial prostatitis in dogs was induced by injection of an E. coli 06 suspension into a branch of the prostatic artery.

Three to six days later, secretion from the inflamed glands was obtained by pilocarpine stimulation and the concentrations of trimethoprim, sulphamethoxazole, erythromycin, doxycycline and ampicillin were measured during constant infusion of these drugs. In the prostatic secretion, only the concentrations of the lipid soluble substances trimethoprim and erythromycin exceeded the corresponding plasma levels. These two substances may therefore be of value in the treatment of bacterial prostatitis.

Key words: Antimicrobials - Experimental bacterial prostatitis.

The antimicrobial treatment of acute and chronic bacterial prostatitis is still unsatisfactory since only very few antimicrobial substances actively concentrate in the prostatic secretion (2-8).

The reason is that any substance, in order to appear in the prostatic secretion at levels equivalent to or higher than those in the plasma has to have certain chemical features: 1) lipid solubility, 2) high pKa, 3) low protein binding in the plasma and, most importantly, 4) the substance has to be a base (5, 7). Among the antimicrobial substances fulfilling these requirements are the basic macrolides (e. g. erythromycin, spiramycin, oleandomycin), and trimethoprim.

Several investigators (2-8) have demonstrated that these substances concentrate in the normal canine prostatic tissue and secretion as well as in human prostatic tissue. Concentrations of several antibiotics have also been investigated in the prostatic secretion of a patient with cutaneous ureterostomy, thus excluding urinary contamination of the prostatic secretion (4, 5).

All previous dog experiments and most of the investigations in humans were carried out in normal prostatic glands, or in patients with benign hyperplasia of the prostate or prostatic cancer. Therefore, we do not know if these mechanisms are the same in the presence of inflammatory processes in the prostate. Many authors concluded that in acute bacterial prostatitis any antibacterial substance with sufficient activity against the specific pathogens will be effective in treatment, regardless of chemical features. However, in chronic bacterial prostatitis, practically no antibiotic or chemotherapeutic agent has been proven to be satisfactory for long-term treatment.

In order to develop guidelines for the antimicrobial treatment of acute bacterial prostatitis, the concentrations of various antibacterial substances in the prostatic secretion of dogs with experimental bacterial prostatitis were tested and compared to the results of previous experiments with healthy dogs.

MATERIAL AND METHODS

Sexually mature male mongrel dogs were anaesthetized with intravenous Pentothal. The prostate gland was exposed through a para-

Table 1. Dosages of various antimicrobial substances

	Bolus (mg/kg)	Constant infusion (mg/kg/h)
Erythromycin	3.5	8
Trimethoprim	4	0.5
Sulfamethoxazole	20	2.5
Ampicillin	3	10
Doxycycline	10	0.5

median incision just proximal to the symphysis pubis. The bladder neck was transected and the bladder was closed, thus isolating it from the prostate and excluding urine contamination. The proximal end of the urethra was also closed thus allowing secretion to drain distally only.

The vasa deferentia were transected to prevent possible admixture of semen. A plastic cystostomy tube was inserted into the dome of the bladder through the abdominal muscles for urinary drainage.

Pure prostatic secretion could now be obtained via an urethral catheter by prostatic massage or - if necessary - after stimulation with pilocarpine 0.25 mg/kg i. v.

One major branch of the prostatic arteries was now freed from its fascial sheath and the blood flow interrupted temporarily by application of a tourniquet. A 25 gauge needle with a silastic tube extension was used to cannulate the artery and 0.5 ml of a suspension of approximately 10^6/ml E. coli 06 was injected. After withdrawal of the needle and release of the tourniquet the resulting bleeding at the site of the injection was stopped by application of a fibrin sponge. To prevent spread of the infection in the periprostatic tissue, a freshly prepared penicillin solution (3,000,000 units per ml) was flushed over the surrounding tissue and the wound was closed in layers.

Before and after introduction of the infection, bacterial cultures were obtained from the prostatic secretion to confirm the establishment of a bacterial prostatitis.

Three to six days later, five commonly used antibacterial substances (erythromycin, trimethoprim, sulphamethoxazole, ampicillin and doxycycline) were tested. The compounds were given as a bolus intravenously, followed by a constant intravenous infusion. The dosages (mg/kg) of the various substances are listed in Table 1.

Blood samples were drawn from a cannulated leg vein before and directly after injection of the bolus dosage and at 30 min intervals there-

Table 2. Antimicrobial substances in prostatic secretion (PS) and plasma (Pl) in dogs with and without prostatitis (mean ± 1 SD, range in brackets)

	Normal dogs					Prostatitis dogs					
Drug	No. of dogs	Plasma	Prostatic secretion	Ratio $\frac{PS}{Pl}$	pH in PS	No. of dogs	Plasma	Prostatic secretion	Ratio $\frac{PS}{Pl}$	Difference between ratios of normal and infected dogs	pH in PS	Difference between pH of normal and infected dogs
Erythromycin	4	7.1 ± 3.3 (1.6 - 14.5)	8.9 ± 5.3 (1.7 - 5.2)	1.5 ± 1.1 (0.3 - 5.2)	6.6 ± 0.2	4	6.6 ± 2.6 (1.7 - 11)	14.8 ± 11.3 (0.8 - 50)	2.5 ± 1.4 (0.2 - 5.5)	($p < 0.001$)	6.4 ± 0.2	($p < 0.05$)
Trimethoprim	6	3.6 ± 1.8 (1 - 8.9)	28 ± 1.5 (6.5 - 60)	9.1 ± 4.2 (2.5 - 17.6)	6.5 ± 0.3	3	2.6 ± 1.0 (0.6 - 5.5)	31.1 ± 10.9 (3.9 - 53)	11.5 ± 4.6 (2.8 - 21.2)	($p < 0.1$)	6.4 ± 0.2	($p < 0.1$)
Sulphamethoxazole	3	30 ± 3.6 (15.5 - 42)	4.2 ± 0.8 (2.8 - 6.3)	0.13 ± 0.16 (0.08 - 0.32)	6.5 ± 0.3	3	54.6 ± 14.7 (30 - 102)	3.8 ± 1.6 (0.7 - 7.6)	0.07± 0,03) (0.03- 0.14)	($p < 0.001$)	6.4 ± 0.2	($p < 0.05$)
Ampicillin	3	27 ± 8 (17 - 50)	1.0 ± 0.4 (0.2 - 1.2)	0.04 ± 0.02 (0.01 - 0.1)	N. A.	2	26.5 ± 12.4 (10 - 56)	< 0.1	-		6.5 ± 0.2	
Doxycycline[a]	3	18 ± 4.5 (11 - 25)	2.3 ± 0.6 (2 - 3)	0.16	N. A.*	2	4.4 ± 2.7 (1.6 - 12)	1.3 ± 0.3 (0.6 - 2)	0.4 ± 0.3 (0.05- 0.9)		6.6 ± 0.2	

[a] *Results for normal dogs: Stamey, T. A. (7)*

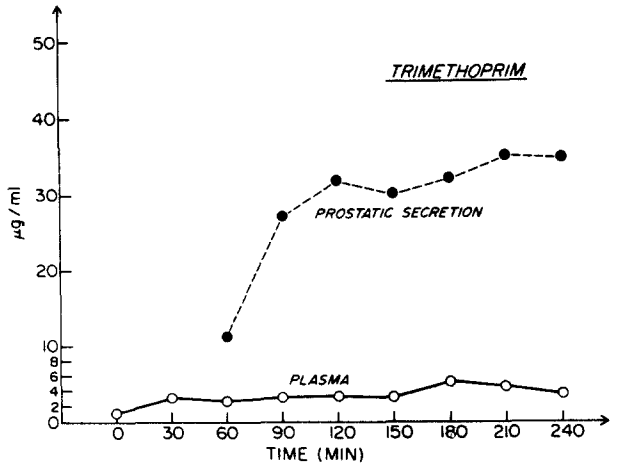

Fig. 1. Concentration of trimethoprim in canine prostatic secretion compared to plasma levels after induction of experimental bacterial prostatitis

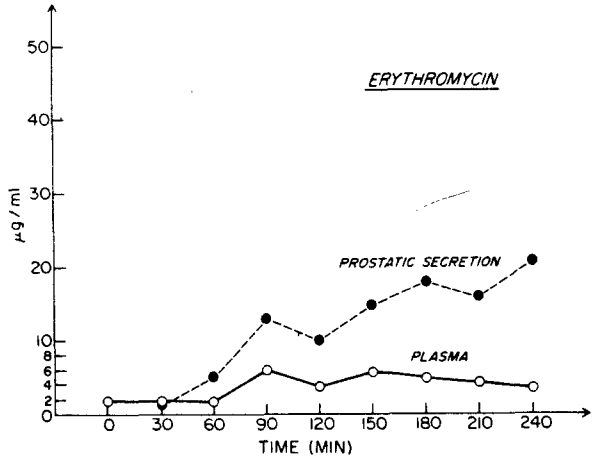

Fig. 2. Increased prostatic secretion concentration of erythromycin in comparison to the plasma levels after induction of experimental bacterial prostatitis in a dog

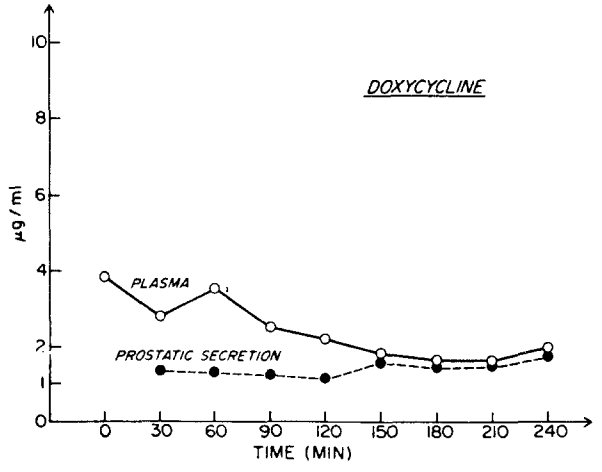

Fig. 3. Prostatic secretion levels of doxycycline in comparison to the plasma concentrations in a dog with experimental bacterial prostatitis

after for 4h. Prostatic secretion was stimulated with pilocarpine 0.25 mg/kg i. v. and samples were collected at the same intervals via the catheterized urethra. The pH of the prostatic secretion was determined hourly.

All samples were frozen immediately and stored at -17° C until bioassayed.

Bioassays were performed using Sarcina lutea ATCC 9341 for erythromycin and ampicillin, B. pumilus CN607 for trimethoprim, and B. cereus ATCC 1174 8-3 for doxycycline. The Bratton-Marshall chemical method was used for sulphamethoxazole determination (1).

The pH determinations were carried out on a BMS3 MK2 blood micro system. To calculate the concentration ratios between prostatic secretion and plasma, the average plasma concentration was used, calculated from the values measured at the beginning and at the end of each 30 min period. A Student t-test was used to determine the significance of the differences in ratios between animals with normal and infected prostates.

RESULTS

After injection of bacteria into the prostatic artery all dogs developed signs of acute prostatitis as determined by repeated rectal palpation.

All but two dogs had positive bacterial cultures for E. coli 06 in their prostatic secretion whereas none had a positive culture prior to injection of the bacteria. The two dogs with negative prostatic secretion cultures showed signs of acute prostatitis and subsequent cultures taken directly from the prostatic tissue were positive. The histological picture also confirmed the successful development of a prostatitis.

The overall results of the studies with the various antimicrobial substances are listed in Table 2. Since ampicillin levels in the secretion of the inflamed prostate are too low for bioassay determination, no ratios could be calculated and no statistical analysis carried out. The test results for doxycycline in normal dogs were taken from Stamey (7). Since these results were obtained from experiments carried out by injection of a single dose of antibiotics in contrast to our constant infusion technique, a direct comparison with our results is not possible.

The mean pH value in the prostatic secretion before and after inflammation increased slightly from 6.27 $\pm$ 0.27 ($\pm$ 1 SD) to 6.58 $\pm$ 0.21, but the increase is not statistically significant ($p = 0.1$). The pH changes for the different antibiotic studies are listed together with the figures for the antibiotic levels. Here the picture is quite different and in some cases a statistically significant decrease in the pH values is observed.

Figures 1 to 3 demonstrate typical examples of experiments with trimethoprim, erythromycin and doxycycline respectively in dogs with prostatitis.

DISCUSSION

The levels of the various antibiotics tested in dogs with and without prostatitis and the ratios between prostatic secretion and blood level are interesting. Only those substances which are concentrated in the normal prostatic secretion also appear in high levels in the secretions of the acutely inflamed glands. Whereas ampicillin is not detectable at all, doxycycline and sulphamethoxazole are only secreted in very low concentrations. This may explain the frequent treatment failures in prostatitis with these substances.

As in normal prostates, only basic substances appear in concentrations higher than the corresponding plasma levels, probably since basic substances will be more ionized in the acid prostatic secretion than in the alkaline plasma (8). The lower the pH of the prostatic secretion, the higher the concentration of a basic substance in the prostatic secretion will be. Two of our five antibacterial substances (erythromycin and trimethoprim) are bases and for both we found higher prostatic secretion/plasma ratios accompanying a decrease in the pH. On the other hand, sulphamethoxazole, an acid, was less concentrated in infected prostatic secretion. Ampicillin, is a lipid insoluble substance and therefore practically absent from prostatic secretion.

Doxycycline is an amphoteric substance with three different pKa values. Apparently a different diffusion mechanism becomes involved in this case since our doxycycline tests gave much higher ratios in inflamed glands than Stamey found in normal ones (7). But these two groups are not directly comparable since Stamey used a single injection technique. It seems logical that for the antimicrobial treatment of acute bacterial prostatitis only those substances can be expected to be effective which also concentrate in the secretion of the prostate gland. From the clinical point of view, an infection with gram-positive bacteria could be treated with erythromycin whereas infections with gram-negative organisms warrant a trial with trimethoprim which presently is available only in combination with sulphamethoxazole. Ampicillin seems to be of no value at all whereas doxycycline could be effective if given in high doses.

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