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Antimicrobial Substances in Secretion, Interstitial Fluid, and Tissue of Normal and Infected Canine Prostate Glands

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Summary. Seven antimicrobial substances – three basic, three acidic and one amphoteric – were given in constant infusion experiments to dogs to monitor their distribution in the prostate gland. In some of the dogs an experimental bacterial prostatitis had been induced prior to the experiments. Drug levels were measured in plasma (Pl), prostatic interstitial fluid (PIF), prostatic secretion (PS) and prostatic tissue (PT). Drug levels in PIF differed considerably from those in PS. In PS and PIF only basic substances exceeded the corresponding plasma levels. Concentrations of acidic substances in PS and PIF never exceeded the simultaneous plasma levels. In PIF the concentration of these drugs was significantly higher than in PS. Our results show that previous studies of prostatic secretion levels only were too optimistic for alkaline drugs and too pessimistic for acidic drugs.

Key words: Experimental prostatitis, Interstitial fluid, Antimicrobials.

Introduction

The successful antimicrobial treatment of inflammatory processes depends, in addition to selection of an active antibacterial substance, on the concentration of the drug at the site of the bacterial invasion.

In the study of bacterial prostatitis, many authors compared drug levels in prostatic secretion to the corresponding plasma levels and concluded that only lipid soluble bases with a high pKa and low protein binding could exceed the corresponding plasma levels [2, 6–9].

This study presents a new concept regarding drug concentrations in normal and infected canine prostate glands. In order to assess the "true" concentrations of commonly used antimicrobials, two animal research models were combined – a model of an experimental bacterial prostatitis in dogs [1] and the implantation of tissue chambers into normal and infected glands [5]. These techniques made it possible to compare the concentrations of various groups of antimicrobials at the site of the inflammatory process (namely the interstitial spaces as shown in our experiments with experimental prostatitis), in contrast to previous reports concerning drug levels in plasma or prostatic secretion only. We report the investigation of seven substances that illustrate the relative importance of lipid solubility, pKa and pH of prostatic compartments.

Material and Methods

Tissue chambers were implanted through a suprapubic paramedian incision into the prostates of twenty sexually mature, male, mongrel dogs, weighing 13–28 kg. Two chambers per dog were placed, one into each lateral lobe. Pentothal i.v. was used for anaesthesia. Following vasectomy and closure of the bladder neck with a rubber tourniquet, a 14 F disposable urethral catheter was used to collect samples of prostatic secretion (PS). All baseline PS-samples were cultured for bacterial growth.

The tubes of the tissue chambers were placed subcutaneously to make repeat access to these tubes feasible.

After 4 to 5 weeks, an experimental bacterial prostatitis was created in six of the 20 dogs as described elsewhere [1]. Briefly summarised, a 0.5 ml suspension of approximately 10^6 E. Coli 06 bacteria was injected slowly into one branch of the prostatic arteries.

The studies of the antibacterial compounds were performed in the dogs 4 to 5 weeks following implantation of the chambers or creation of the experimental prostatitis respectively. The compounds investigated and their bolus and constant infusion dosages are listed in Table 1.

The drugs investigated include three bases (trimethoprim and the two macrolides erythromycin and rosamicin), each with good lipid solubility and relatively high pKa's and three acids (ampicillin, netilmicin, sulfamethoxazole). Ampicillin is practically lipid-insoluble, whereas the other two acid drugs tend to be fat soluble.

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Table 1. Antibacterial compounds investigated

Name	Bolus intravenously (mg/kg)	Constant infusion intravenously (mg/kg/h)
Ampicillin	3	10
Doxycycline	10	1
Erythromycin	10	3
Netilmicin	10	3
Rosamicin	10	3
Sulfamethoxazole	20	2.5
Trimethoprim	4	0.5

Doxycycline is a member of the tetracycline group and has amphoteric characteristics with three pKa's and good lipid-solubility. After the bolus, the compound was given by constant infusion [2, 6, 7]. The infusion was calculated so that the bolus would be infused over a period of approximately three to four serum half-lives of the compound. Samples of plasma (PI) from a cannulated femoral vein, PS (following stimulation with 0.25 mg/kg pilocarpine intravenously) and of prostatic interstitial fluid (PIF) from the tissue chamber were obtained shortly before and after application of the bolus and every 30 min thereafter for up to 4 h.

All samples were immediately frozen and stored at −17 °C until bioassay determination. pH values of the prostatic secretion were measured hourly on a PHM 72 MK 2 Digital Acid-Base Analyzer (Radiometer, Copenhagen).

Bioassays of the antibiotics were performed by a disc diffusion method using Sarcina lutea ATCC 9341 for erythromycin and ampicillin, Bacillus pumilus CN 607 for trimethoprim, B. cereus ATCC 1174 8–3 for doxycycline, Bacillus subtilis for rosamicin and netilmicin. Sulfamethoxazole concentrations were determined by the Bratton-Marshall Method [2].

Serum standards were prepared with pooled canine serum, prostatic secretion standards with pooled canine prostatic secretion and standards for prostatic interstitial fluid by dilution with phosphate buffer, pH 8.

For easier comparison between the individual groups of chemically different substances, the ratios of PS and PIF to PI were calculated using the median between the two values measured at the beginning and at the end of each 30 min sampling period in each animal.

To compare ratios of PIF/PI and PT/PI the ratios obtained at the end of each drug study were used and compared to the PT/PI ratios obtained in previous dog studies performed by identical technique [2, 6, 7].

In several dogs, two studies were performed at an interval of at least one week.

The Student's *t*-test was used for statistical analysis and comparison among the various compounds.

Table 2. Concentration ratios of various drugs in prostatic interstitial fluid, prostatic secretion and prostatic tissue over plasma, and pH in prostatic secretion in dogs (Mean ± 1 S.D., [Rangel])

Drug	No. of Dogs normal (n) or infected (i) prostates	Ratios			pH in Prostatic Secretion
		Interstitial fluid plasma	Secretion plasma	Tissue plasma	
Rosamicin	2 n	3.4 ± 0.8 (2.1–6.7)	7.6 ± 1.9 (6.7–12.9)	3.4 ± 1.2[a] (1.8–4.5)	6.4 ± 0.2 (6.0–6.7)
	2 i	4.3 ± 1.6 (1.5–9.0)	10.2 ± 2.8 (6.5–14.5)		6.6 ± 0.2 (6.4–6.8)
Erythromycin	2 n	1.9 ± 0.6 (1.0–2.9)	3.9 ± 0.8 (3.1–5.7)	1.7 ± 1.0[a] (1.0–3.2)	6.5 ± 0.1 (6.3–6.7)
	3 i	2.8 ± 1.2 (0.8–6.2)	4.0 ± 2.3 (1.0–9.1)		6.3 ± 0.4 (5.7–6.8)
Trimethoprim	3 n	6.7 ± 3.8 (1.3–12.5)	14.8 ± 7.7 (2.9–32.8)	4.5 ± 3.5[b] (2.0–7.7)	6.6 ± 0.5 (5.9–7.5)
	2 i	6.3 ± 2.6 (0.8–11.7)	10.1 ± 3.2 (4.6–16.7)		7.1 ± 0.3 (6.5–7.6)
Sulfamethoxazole	2 n	0.43 ± 0.25 (0.16–1.06)	0.18 ± 0.06 (0.08–0.3)	0.68 ± 0.26[b] (0.39–0.89)	6.9 ± 0.4 (6.3–7.5)
	2 i	0.51 ± 0.26 (0.01–0.85)	0.18 ± 0.08 (0.07–0.37)		7.1 ± 0.3 (6.6–7.6)
Doxycycline	3 n	0.72 ± 0.12 (0.47–1.04)	0.59 ± 0.1 (0.4–0.8)	0.67 ± 0.11 (0.55–0.77)	6.9 ± 0.2 (6.6–7.2)
Ampicillin	4 n	0.17 ± 0.01 (0.02–0.35)	0.017 ± 0.01 (0.002–0.05)	0.09 ± 0.009 (0.08–0.1)	6.6 ± 0.3 (6.1–7.0)
Netilmicin	2 n	0.35 ± 0.18 (0.07–0.7)	0.03 ± 0.03 (0.01–0.13)	–[c]	6.7 ± 0.3 (6.3–7.1)

[a] Data for four dogs from Ref. 1
[b] Data for six dogs from Ref. 6
[c] Dogs were kept alive after experiment

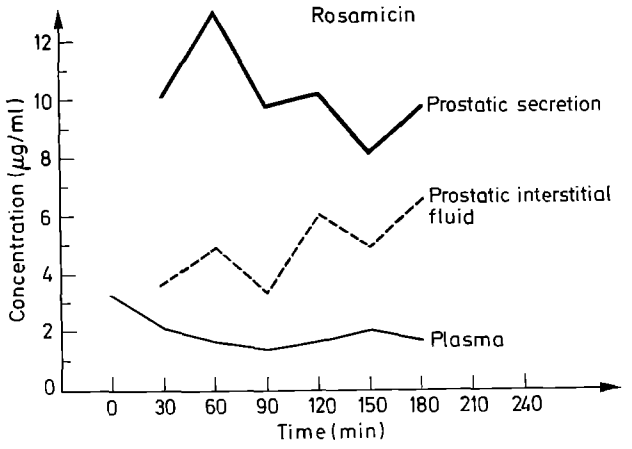

Fig. 1. Rosamicin concentrations in canine plasma prostatic secretion and interstitial fluid during constant infusion

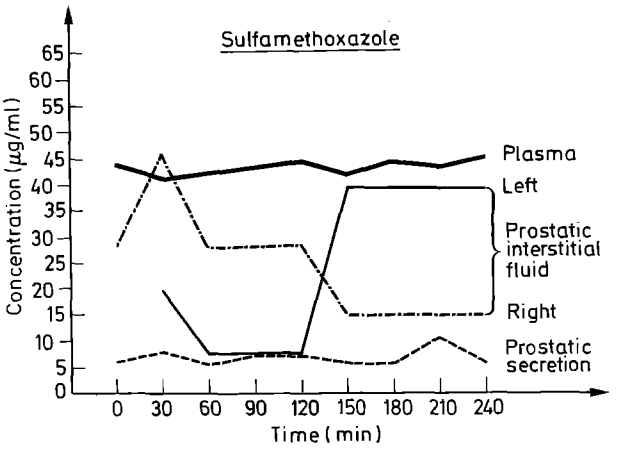

Fig. 4. Sulfamethoxazole concentrations in canine plasma, prostatic secretion and interstitial fluid during constant infusion

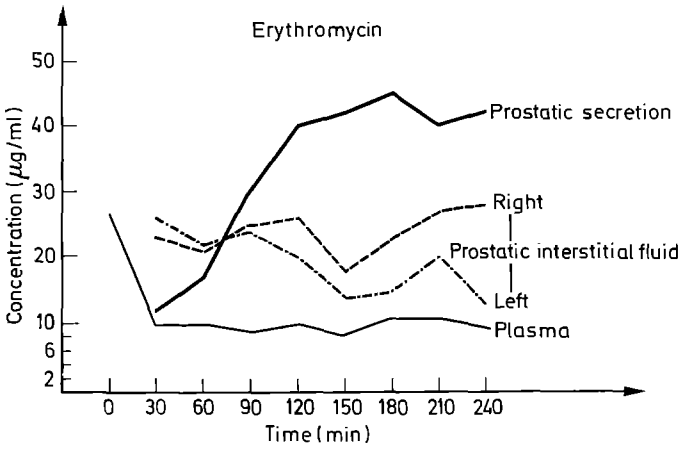

Fig. 2. Erythromycin concentrations in canine plasma, prostatic secretion and interstitital fluid during constant infusion

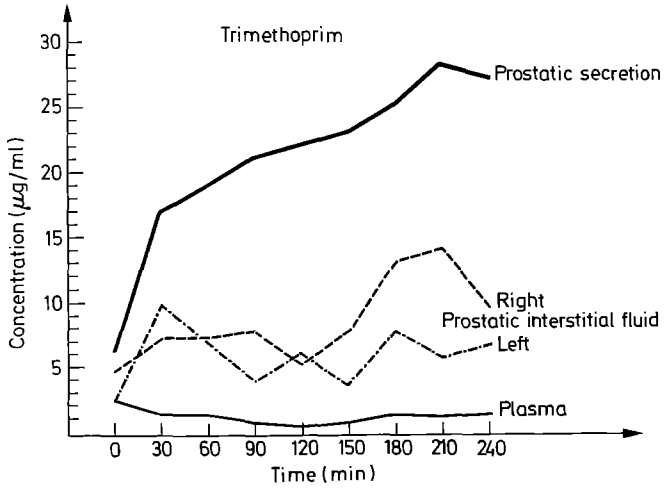

Fig. 3. Trimethoprim concentrations in canine plasma, prostatic secretion and interstitial fluid during constant infusion

The successful creation of a bacterial prostatitis was confirmed by bacterial cultures from prostatic secretion and interstitital fluid as well as histologically in those dogs sacrificed after the experiments.

Results

All but one implanted tissue chamber healed in completely and during the infusion studies approximately 0.01 ml of a clear, yellowish fluid could be aspirated every 30 min. No case showed macroscopic admixture of fresh blood. Contamination with urine was excluded by application of a tourniquet around the bladder neck and instillation of the suprapubic cystostomy. Creatinine determinations in 10 randomly selected samples of prostatic secretion were negative confirming the lack of urine contamination.

The establishment of an experimental bacterial prostatitis was confirmed by positive bacterial cultures for E. coli from the prostatic secretion, prostatic interstitital fluid or histologically. It is of note that in one case no bacterial growth from the prostatic secretion could be observed whereas, in this case, prostatic interstitial fluid cultures showed heavy growth and histological examination confirmed the induction of a prostatitis.

Table 2 summarises the overall results. The results from the studies in dogs with experimentally infected prostates are listed separately from the values obtained in normal dogs.

There was no statistically significant difference between concentration ratios in prostatic interstitial fluid and prostatic tissue for any of the drugs studied, a finding of great clinical importance. The results for the individual antibacterial compounds showed that drug levels in the prostatic interstitial fluid were between plasma and prostatic secretion concentrations.

Concentration ratios of PIF/Pl for dogs with normal and infected prostates were significantly different only for erythromycin ($p < 0.001$), whereas for rosamicin, trimethoprim and sulfamethoxazole there were no significant differences.

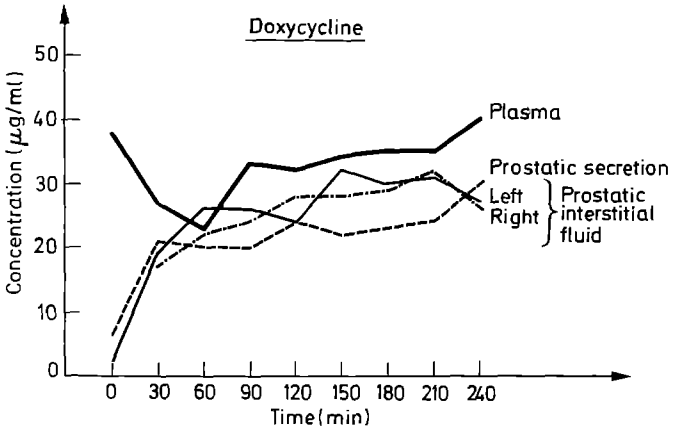

Fig. 5. Doxycycline concentrations in canine plasma, prostatic secretion and interstitial fluid during constant infusion

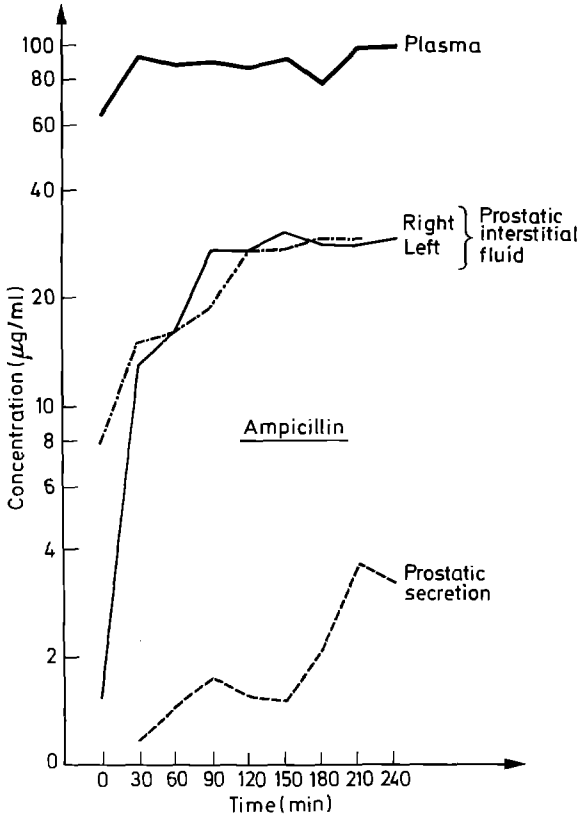

Fig. 6. Ampicillin concentrations in canine plasma, prostatic secretion and interstitial fluid during constant infusion

Figures 1 to 7 represent typical examples of each test compound.

Discussion

This ist the first systematic study of drug concentration ratios in the prostate gland at the site of an inflammatory process. Previous studies were done either by using single

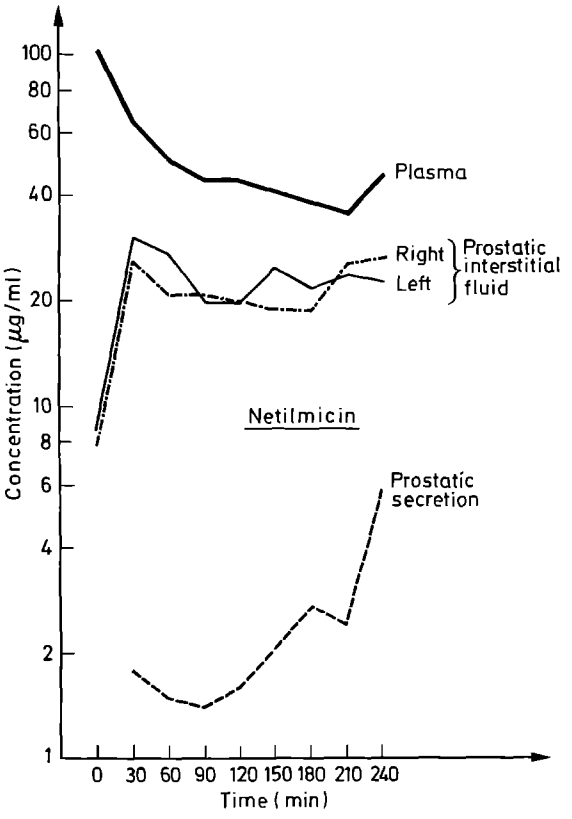

Fig. 7. Netilmicin concentrations in canine plasma, prostatic secretion and interstitial fluid during constant infusion

injections [8] or by investigating one or two compounds. By using a constant infusion technique, almost constant blood levels were established over a period of several hours, thus enabling a comparison of drug levels in various fluids. Delays in equilibration can be excluded by this method.

It was not the purpose of this study to reach bacteriostatic or bacteriocidal concentrations.

It is evident form the results with tissue chambers that only basic drugs exceed the corrresponding plasma levels. Nevertheless, the levels in prostatic interstitital fluid are considerably lower than the corresponding values for prostatic secretion.

Therefore, a drug which is highly concentrated in the secretion of the prostate gland will not necessarily reach the same high levels at the site of bacterial invasion, the interstitium.

On the other hand, all acidic drugs were less concentrated in prostatic secretion than in plasma. However, acidic drug levels in the interstitial spaces were higher than those in the prostatic secretion so that even an acidic drug might be of therapeutic value if administered in a high enough dose.

Pilocarpine stimulation caused a definite increase in the output of prostatic secretion but the flow rate of the interstitial fluid did not change so that an admixture between the two fluids seems unlikely.

The fact that the concentration ratios between normal and experimentally infected glands in dogs showed no differences may well be explained by the development of a chronic nonspecific inflammatory reaction around the tissue chambers.

Another important result of this study is that we could not show significant differences between interstitial fluid and whole tissue drug concentration ratios. This means that when studying a compound in humans – where interstitial fluid cannot be obtained – drug determinations in prostatic tissue (e.g. obtained during prostatectomy) will give a good indication of drug levels at the site of an inflammatory process.

It is apparent from this study that the investigation of drug levels in prostatic secretion alone is not applicable in the treatment of bacterial prostatitis and can lead to either too optimistic conclusions for basic compounds or to too pessimistic an estimate for acidic compounds.

This study was carried out in dogs and caution is advised concerning clinical application. It is suggested that a drug used in the treatment of bacterial prostatitis should be a base with a high pKa and good lipid solubility as well as low protein binding. The duration of treatment – in chronic cases in particular – should be extended. If bacterial susceptibility suggests the use of an acidic substance, high dosages over extended periods can be tried, especially with drugs which penetrate tissue well. If a combination drug is used, an additional dosage of the acidic compound is recommended.

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