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Penetration of Antibiotics into the Surgical Wound in a Canine Model

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The dose and timing of antimicrobial agents given for surgical wound prophylaxis should be based on the concentration-time profile of the drug in tissue at the site of contamination. However, concentrations of antimicrobial agents in surgical wounds are difficult to determine accurately. Since a surgical wound is a unique extravascular compartment with increased vascular permeability and a high surface area/volume ratio, antibiotic concentrations in sera and surgical wounds should be similar. To test this hypothesis, the pharmacokinetics of single intravenous doses of cefazolin (40 mg/kg) and gentamicin (4 mg/kg) in sera and surgical wounds in a clinically relevant surgical model using dogs were compared. Drug concentrations were determined in interstitial fluid in muscle biopsies taken randomly from wound surfaces and serial wound fluid samples collected after the incisions were closed. Protein binding of cefazolin and gentamicin in sera and wound fluids was low (≤29 ± 9%) in this canine model. Cefazolin and gentamicin equilibrated rapidly (≤30 min) between serum and the surgical wound, and concentrations in the two sites declined in parallel. Values for the area under the concentration-time curve, mean residence time, and terminal half-life in serum and the surgical site for each drug were similar. Cefazolin concentrations in serum underestimated the time during which concentrations in surgical wounds exceeded the susceptibility breakpoint MIC for important pathogens by an average of 58 min (range, 26 to 109 min; $P = 0.036$); for gentamicin, the underestimation averaged 30 min (range, 10 to 60 min; $P = 0.036$). These data support the concept that the concentration-time profiles of antimicrobial agents in serum may prove valuable clinically as guides to determining the dose and timing of antibiotic administration necessary for effective antimicrobial prophylaxis in surgery. Further studies are needed to determine the surgical wound pharmacokinetics of highly protein-bound antibiotics.

For effective antimicrobial prophylaxis, antibiotics must be present in tissue at the surgical site throughout the operation in concentrations sufficient to prevent growth of contaminating pathogenic microorganisms (11). Clinical trials in surgical patients have shown that prophylaxis can fail if the antibiotic dose is too low or the half-life in tissue is too short (5, 10, 14). The dose and timing of antibiotic administration for prophylaxis should be based on the concentration-time profile of the drug in the surgical wound. Unfortunately, concentrations of antibiotics at surgical sites are difficult to determine accurately. Levels of antibiotics in serum, however, are known or easily measured. We hypothesized that since the trauma of surgery alters blood vessel permeability in the large surface area of a surgical wound, antibiotic concentrations in tissue interstitial fluid and wound fluid in a surgical wound are similar to the levels in serum. To test this hypothesis, we developed a canine surgical model to obtain multiple samples of muscle tissue from a wound surface and fluid from the wound after closure. We chose to study cefazolin and gentamicin, two widely used antibiotics with different properties. Single intravenous (i.v.) dose concentrations of cefazolin and gentamicin were measured in surgical site muscle interstitial fluid, wound fluid, and serum to determine the rate and extent of delivery of these antimicrobial agents to surgical wounds.

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MATERIALS AND METHODS

Surgical model. Twelve healthy adult mixed-breed dogs (14 to 24 kg) of both sexes were divided into two equal groups. After food was withheld overnight, all dogs received acepromazine (0.1 mg/kg intramuscularly) as preanesthetic medication. Anesthesia was induced with i.v. thiamyal sodium and maintained with halothane and oxygen administered by a closed-circle system with intermittent positive-pressure ventilation. Lactate-treated Ringer solution (10 to 20 ml/kg per h) was administered i.v. for the duration of the experiment. Foreleg amputation was done by transecting the extrinsic muscles of the scapula, and the wound was closed by a standard technique. This is a unique model of an actual surgical wound affected by the inflammatory mediators released during a major surgical procedure. All dogs were maintained in a surgical plane of general anesthesia throughout the study. The dogs were continuously monitored and euthanized by i.v. injection of concentrated barbiturate solution after the last sample was collected. This study was approved by the Animal Care Committee, School of Veterinary Medicine, University of Wisconsin, Madison.

Cefazolin (40-mg/kg i.v. bolus) or gentamicin (4-mg/kg i.v. bolus) was given to a group of dogs ($n = 6$ each) at the start of surgery. Blood samples were obtained immediately before (time zero), at 5, 15, 30, and 45 min, and 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after the drugs were administered. The samples were collected in sterile tubes, allowed to clot at room temperature for 1 h, and centrifuged at 1,300 × g for 10 min, and the serum was stored in plastic storage tubes at −70°C until assayed.

During the surgical procedure, sequential muscle biopsies

(250 to 500 mg) were obtained randomly from wound surfaces immediately before (time zero) and at 5, 15, 30, 45, and 60 min (time of wound closure) after the drugs were administered. Muscle samples were minced and stored in plastic storage tubes at −70°C until assayed. On the day of assay, the muscle samples were weighed and a volume of buffer equal to twice the muscle weight was added. The sample was cooled with ice while homogenized and centrifuged at 1,300 × *g* for 10 min, and the supernatant was collected for assay.

Wound fluid samples (1.5 ml) were obtained at 1.5, 2, 2.5, 3, 4, 5, and 6 h after the drugs were administered. Wound fluid was collected through a fenestrated silastic tube (4-mm diameter) placed into the interior of the surgical site, brought through a stab wound ventral to the incision, and connected to a 50-ml syringe. Wound fluid was evacuated by constant suction applied to the silastic tube via the syringe. Every 15 min, the wound was manually compressed to ensure that fluid was continuously evacuated. Any fluid collected between sampling times was discarded. Wound fluid samples for assay were collected by manual aspiration for 3 to 5 min with a disposable 3-ml syringe. Samples were placed in sterile tubes and centrifuged at 1,300 × *g* for 10 min, and the supernatant was stored at −70°C until assayed.

Antibiotic assay. Antibiotic concentrations were determined by a modified agar plate diffusion technique (2). *Staphylococcus aureus* and *Bacillus subtilis* were used as test organisms for cefazolin and gentamicin, respectively. Each sample was analyzed in duplicate. The antibiotic concentration corresponding to the mean of the two measurements was determined by comparing these diameters with those of various dilutions of a standard (prepared in pooled normal canine serum) by linear regression analysis. Pilot studies showed that standard curves calculated on the basis of reference standards prepared in canine serum, muscle homogenate supernatant, or wound fluid were identical. Standards were included on each assay plate to compensate for plate-to-plate variation. The correlation coefficient for the standard curves prepared for all experiments was greater than 0.98. The mean curve coefficient of variance was 6.3%. The sensitivity of the assay for cefazolin was 0.39 μg/ml, and that for gentamicin 0.195 μg/ml.

Calculation of the antibiotic concentration in muscle interstitial fluid was based on the premise that cefazolin and gentamicin are hydrophilic antibiotics with poor intracellular penetration (1, 9, 12, 15). Thus, the antibiotic present in muscle samples was located primarily in the extracellular compartment, i.e., plasma and interstitial fluid. The volume of interstitial fluid was estimated as equal to the extracellular space minus the plasma volume. The extracellular space calculated by the inulin method in mammalian skeletal muscle is approximately 17% of the muscle volume (8). Hemoglobin levels in blood and muscle homogenate supernatant were used to correct for the antibiotic concentrations present in plasma in the muscle samples. Hemoglobin in muscle homogenates was determined by the cyanomethemoglobin procedure (13), and that in blood was determined by photometric measurement (Coulter Counter, model S7-70; Coulter Electronics, Inc., Hialeah, Fla.). The antibiotic concentration in interstitial fluid (C_i) in the muscle samples was calculated by the following formula: $C_i = C_t - [C_t \times (Hb_t/Hb_b)]/0.17$, where C_t is the antibiotic concentration in muscle homogenate supernatant (corrected for dilution in buffer), Hb_t is hemoglobin in muscle homogenate, and Hb_b is hemoglobin in blood.

Protein binding. The protein binding of cefazolin and gentamicin was determined for serum and wound fluid by ultrafiltration with an MPS-1 unit with YMT membranes (Amicon Corp., Danvers, Mass.). Each antibiotic was added at concentrations of 6.25, 12.5, and 25 μg/ml to each fluid from two control dogs that underwent the same surgical procedure. Samples were allowed to reach equilibrium before ultrafiltration. Samples from the known concentrations in each fluid were centrifuged at 1,200 × *g* for 1 h at 37°C, and the ultrafiltrates, along with the original unfiltered samples of known concentrations, were run in duplicate in the antibiotic assay. The percentage of drug bound was calculated as [(total drug − unbound drug)/total drug] × 100. Differences in binding in the two media were analyzed by Student's *t* test. At 1 and 5 h after completion of surgery, serum and wound fluid total protein and albumin were measured by the colorimetric and bromcresol green methods (Encore; Baker Instrument Corp., Allentown, Pa.), respectively. In addition, the microtiter MICs of cefazolin and gentamicin were determined for *S. aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 in supplemented Mueller-Hinton broth, serum, and wound fluid.

TABLE 1. Mean total protein and albumin concentrations in serum and surgical wound fluid

Parameter	Mean total concn (g/dl) in serum/wound fluid at:	
	1 h after surgery	5 h after surgery
Total protein	4.4/4.3	4.3/3.2
Albumin	2.2/2.1	2.0/1.6

Pharmacokinetics. Pharmacokinetic analysis (7) of antibiotic concentrations was performed for each dog separately. Levels in muscle interstitial fluid and wound fluid were

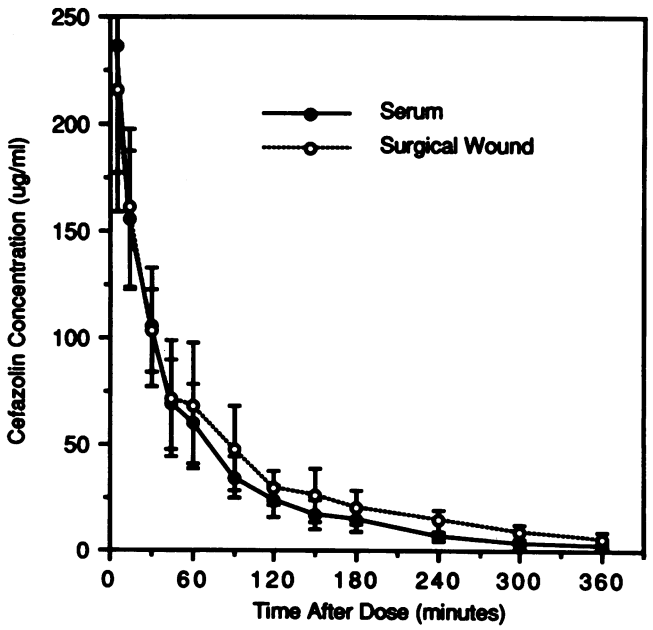

FIG. 1. Concentration-time curves for cefazolin in serum and a surgical wound after a single 40-mg/kg i.v. bolus in a canine foreleg amputation model. Cefazolin concentrations in surgical wounds were determined in interstitial fluid in muscle biopsies taken from the wound surfaces and wound fluid collected after closure of the incisions.

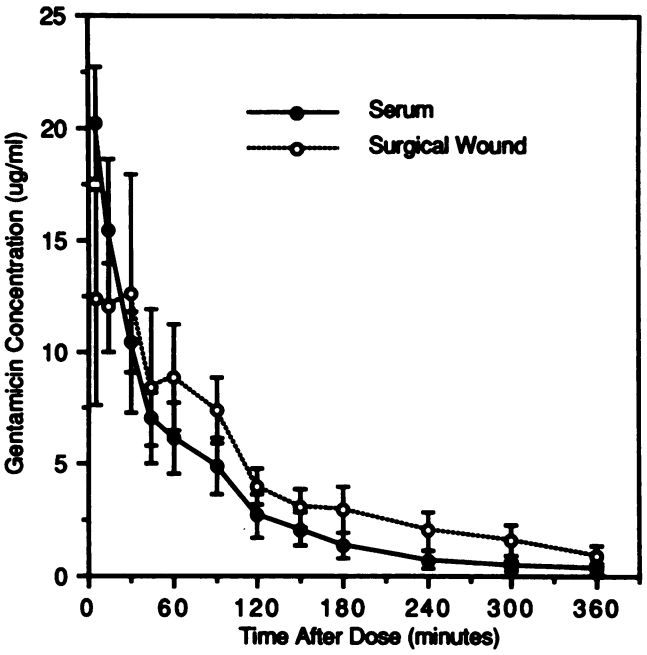

FIG. 2. Concentration-time curves for gentamicin in serum and a surgical wound after a single 4-mg/kg i.v. bolus in a canine foreleg amputation model. Gentamicin concentrations in surgical wounds were determined in interstitial fluid in muscle biopsies taken from the wound surfaces and wound fluid collected after closure of the incisions.

analyzed together and are referred to as surgical wound concentrations. The area under the concentration-time curve (AUC) in serum and at the surgical wound was calculated by the trapezoidal method and extrapolated to infinity. The area from the last concentration point (C_{last}) to infinity was calculated to be C_{last}/β, where β is the negative slope of the terminal portion of the concentration-time curve as estimated by nonlinear least-squares regression analysis (RSTRIP; MicroMath, Inc., Salt Lake City, Utah), assuming a two-compartment model with elimination from the central compartment. The terminal half-life ($t_{1/2\beta}$) was calculated as $0.693/\beta$. The mean residence time (MRT) of an antibiotic in serum and the surgical wound was calculated as AUMC/AUC, where AUMC is the area under the first moment of the concentration-time curve. The time during which the antibiotic concentration in serum and the surgical wound exceeded the susceptibility breakpoint MIC of each drug (R. N. Jones, Antimicrob. Newsl. 5:9–15, 1988) was estimated by using values obtained from regression analysis.

Statistical analysis of paired data in sera versus surgical wounds was performed by using the Wilcoxon rank-sum test. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

The extents of protein binding of gentamicin in canine sera and wound fluids were 29 ± 9 and 11 ± 9%, respectively. For cefazolin, the values were 19 ± 6 and 4 ± 4%. The differences in protein binding between the two drugs were not significant. The total protein and albumin levels in serum and wound fluid at 1 and 5 h after surgery were similar (Table 1). In vitro testing of cefazolin and gentamicin against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 demonstrated similar MICs in broth, serum, and wound fluid. On the basis of these findings, we used total drug concentrations for subsequent pharmacokinetic analysis.

Cefazolin rapidly equilibrated between serum and the surgical wound; the concentrations at the surgical wound paralleled those in serum; i.e., both declined at a biexponential rate (Fig. 1). The rate of gentamicin transfer from serum to the surgical wound was more variable; in three of six animals, a slight delay in equilibration between the concentrations in sera and surgical wounds was observed, but in all animals the difference between the concentrations at the two sites became negligible within 30 min after dosing (Fig. 2).

Comparison the AUC of the drug at the surgical wound with that of the drug in serum showed that the extent of delivery of both agents was excellent; the mean levels of both drugs at surgical wounds were comparable to or slightly higher than those in sera (Tables 2 and 3). The difference between surgical wound and serum AUCs was statistically significant for gentamicin but not for cefazolin.

The MRTs of gentamicin and cefazolin at surgical wounds were significantly longer than those in sera (Tables 2 and 3). This appeared to be due primarily to a slower rate of removal from surgical wounds. A small albeit detectable delay in gentamicin equilibration between sera and surgical wounds may also have contributed to differences between serum and surgical wound MRTs. The mean $t_{1/2\beta}$ for cefazolin in surgical wounds was 104 min compared with 66 min in sera ($P = 0.036$). For gentamicin, the mean $t_{1/2\beta}$ values for surgical wounds and sera were 92 and 64 min, respectively ($P = 0.059$).

A clinically relevant parameter for determining antimicrobial agent dosage for surgical prophylaxis is the time during which concentrations at a surgical wound exceed the MIC for potential pathogens. Cefazolin concentrations in sera underestimated the time during which inhibitory levels were maintained at surgical wounds by an average of 58 min

TABLE 2. Pharmacokinetic parameters of gentamicin in sera and surgical wounds following a dose of 4 mg/kg i.v.

Dog no.	AUC (μg · min/ml)		$t_{1/2\beta}$ (min)		MRT (min)	
	Serum	Wound	Serum	Wound	Serum	Wound
1	1,335.1	2,189.9	83.5	94.7	96.9	145.3
2	1,104.4	1,416.5	78.9	153.7	66.9	143.4
3	1,308.2	1,564.5	63.1	114.5	84.3	170.2
4	1,117.3	1,803.7	56.9	91.4	78.2	129.4
5	1,490.4	2,036.5	67.9	67.0	92.2	124.0
6	858.9	1,182.8	47.2	72.8	56.7	103.5
Mean ± SD	1,202.4 ± 222.1[a]	1,699.0 ± 382.0	66.3 ± 12.4[b]	99.0 ± 28.9	79.2 ± 15.3[a]	136.0 ± 22.6

[a] $P = 0.036$ compared with surgical wound.
[b] $P = 0.059$ compared with surgical wound.

TABLE 3. Pharmacokinetic parameters of cefazolin in sera and surgical wounds following a dose of 40 mg/kg i.v.

Dog no.	AUC (μg · min/ml)		$t_{1/2\beta}$ (min)		MRT (min)	
	Serum	Wound	Serum	Wound	Serum	Wound
1	11,530	17,916	84.4	113.8	89.0	135.4
2	6,322	8,696	61.3	80.7	59.6	80.6
3	13,394	15,669	52.9	89.0	68.6	109.9
4	13,288	12,134	45.6	88.3	62.4	113.7
5	12,233	13,745	63.0	119.7	79.4	114.0
6	12,519	18,204	147.8	174.1	105.8	135.0
Mean ± SD	11,548 ± 2,652[a]	14,394 ± 3,648	75.8 ± 34.3[b]	110.9 ± 31.6	77.5 ± 17.7[b]	114.8 ± 20.2

[a] $P = 0.059$ compared with surgical wound.
[b] $P = 0.036$ compared with surgical wound.

(range, 26 to 109 min; $P = 0.036$); for gentamicin, the underestimation averaged 30 min (range, 10 to 60 min; $P = 0.036$) (Table 4).

DISCUSSION

A surgical wound is a unique extravascular space. The trauma of surgery is a severe injury which causes an immediate and sustained increase in capillary permeability in tissue at the surgical site (16). Closure of the wound creates a compartment with a high surface area/volume (SA/V) ratio. The wound fluid that fills this compartment is a serosanguineous exudate derived from blood plasma as part of the acute inflammation caused by surgery (16). We propose that this fluid, along with the interstitial fluid in tissue at the perimeter of the wound, is the site of antibiotic-microorganism interaction and the location of postoperative wound infection. The concentration of antibiotics in surgical wound extracellular fluid is a critical factor in determining the incidence of wound infection after surgery. The canine model developed for this study was effective and clinically relevant for comparison of antibiotic concentrations in serum and surgical site extracellular fluid in an actual surgical wound during the decisive period when antimicrobial prophylaxis is capable of suppressing the growth of contaminating microorganisms.

Our data show that cefazolin and gentamicin equilibrate rapidly between serum and the extracellular fluid in a surgical wound. Our findings confirm the results of previous studies using artificially created models with a high SA/V ratio (i.e., filter paper disk, cotton thread, and an in vitro kinetic model) which noted that drug concentrations in these fluid spaces tend to parallel changes in serum (3, 6, 17–19). In contrast, drug concentrations in chamber models with a low SA/V ratio (i.e., blister fluid, tissue cage), which are not representative of a surgical wound, have delayed equilibrium, lower peak levels, and prolonged concentrations in comparison with serum (1, 3, 4, 6, 20).

The low protein binding of cefazolin and gentamicin in serum and wound fluid in this canine model may be a factor in the high extent of antibiotic penetration and rapid equilibration between serum and a surgical site. Previous studies using artificial models with a low SA/V ratio have shown that there is a linear relationship between the percentage of protein binding and the extravascular penetration of the antibiotic (18, 21). However, the unique characteristics (i.e., increased vascular permeability and a high SA/V ratio) of a surgical wound suggest that high protein binding would have minimal effect on the penetration of antimicrobial agents into a surgical site.

We conclude that low protein-bound antibiotic concentrations in serum parallel the levels in a surgical wound and levels in serum slightly underestimate the time during which effective levels are maintained at the surgical site. Therefore, the concentration-time profile of antibiotic levels in serum may prove valuable clinically as a guide to determining the dose and timing of antibiotic administration necessary for effective surgical antimicrobial prophylaxis. Further studies are needed to determine the surgical site pharmacokinetics of highly protein-bound antibiotics.

TABLE 4. Times during which drug concentrations exceeded susceptibility breakpoint MICs for important pathogens

Antibiotic	MIC (μg/ml)	Mean (± SD) duration (min) in:	
		Serum	Surgical wound
Cefazolin	16	150 ± 30[a]	208 ± 53
Gentamicin	6	58 ± 14[a]	88 ± 28

[a] $P = 0.036$ compared with surgical wound.

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