Distribution of Systemically Administered Ampicillin, Benzylpenicillin, and Flucloxacillin in Excisional Wounds in Diabetic and Normal Rats and Effects of Local Topical Vasodilator Treatment

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Diabetes mellitus is traditionally associated with an increased risk of complications and infections during wound healing. Diabetes are prone to peripheral vascular disease and peripheral neuropathy, particularly in the lower extremities, which result in loss of sensation and reduced ability to detect injury or developing ulcers. Reduced skin blood flow in the lower extremities in diabetics has been attributed to defects in the function of sensory nerve fibers and subsequent control of the microvasculature compared with normal subjects or controls in both diabetic patients and streptozotocin (STZ)-treated rats (4, 11, 18, 24, 25, 27, 38, 47). Studies examining the effect of various endothelium-dependent and -independent vasodilators have shown that although endothelial function is impaired in diabetes, microvascular smooth muscle responds to normal stimuli (45). It is suggested that subsequent reduced neuronal blood flow induces the observed nerve function deficits. Robertson et al. (33) demonstrated that skin blood flow and nerve conduction velocity in STZ-treated rats at rest were significantly below those of controls, though muscle blood flow appeared normal. They were subsequently able to show that treatment with nifedipine, a vasodilator that acts directly on smooth muscle, could prevent nerve conduction deficits in experimental diabetes. Reduced axon reflex vasodilatation in patients with diabetic neuropathy has been attributed to loss of afferent c-fiber nerve function (20, 29). Stimulation of muscarinic receptors on endothelial cells causes the release of endothelium-derived relaxing factors (13), and studies on vascular smooth muscle have identified nitric oxide as the major endothelium-derived physiologic relaxing agent (28). Westerman et al. (45) examined vasodilator responses in the skin of diabetic patients in response to iontophoresis of the 1% acetylcholine (ACh) (endothelium-dependent) and 1% sodium nitrate (endothelium-independent) vasodilator agents. The studies revealed reduced vasodilator responses to ACh but not to sodium nitrate in patients with diabetic neuropathy compared with normal controls, suggesting that endothelial function in blood vessels is impaired but vascular smooth muscle appears to respond normally. The loss of neuronal function leaves the diabetic unable to detect soft tissue injury in the lower leg and feet and unable to mount any kind of adequate inflammatory response to that injury and, thus, susceptible to aggressive bacterial infection and tissue damage which can be potentially life threatening if not treated effectively.

Clinical reports have confirmed that deep cultures from diabetic soft tissue infections grow multiple organisms and so aggressive therapy with a regimen of broad-spectrum antibiotics is always recommended (23, 35–37). Present treatment of these infections consists of various surgical procedures ranging from debridement to amputation in combination with antibiotic therapy. Initial antibiotic treatment must be bactericidal and most commonly consists of an aminoglycoside employed against aerobic bacteria with a β-lactamase-resistant penicillin to cover enterococci and anaerobic bacteria. In recent years a number of studies have highlighted the problem of relying on serum antibiotic concentrations as an indication of therapeutic levels in soft tissue sites. Seabrook et al. (36) demonstrated in diabetic patients undergoing foot surgery that adequate serum levels of a number of antibiotics were not reflected in infected tissue samples. Storm et al. (41) also showed that following oral administration of cefuroxime in 12 patients, although adequate serum levels were achieved, no antibiotic could be found in wound exudate samples. The inability of systemic antibiotics to penetrate adequately into superficial peripheral wounds and ulcers of diabetics is not surprising considering the numerous reports of reduced blood flow to these regions and the reduced inflammatory and/or immune responses of diabetic patients necessary to overcome continued tissue infestation by bacteria.

The aim of the present study was to take advantage of the impaired microvascular control demonstrated in STZ-treated diabetic rats to create a model of excisional wound healing in which the penetration of a number of commonly used β-lactam antibiotics could be measured. The literature shows that a direct comparison of wound contracture rates with those in non-diabetic animals has never been published using this model. A
second aim was to determine the effect of stimulation of dilation of the local microvasculature on antibiotic penetration into wounds either on both the abdomen and hindleg, using the endothelium-dependent (ACH) and -independent (sodium nitroprusside [SNP]) vasodilator (12) applied directly over the granulating wound site. The hypothesis behind this study is that, as suggested in the literature, the smooth muscle of the microvasculature of diabetes functions normally, then an endothelium-independent vasodilator may cause dilation of vessels in and around the wound site and increase the delivery of systemically administered antibiotics simply because of the increased perfusion of the area.

MATERIALS AND METHODS

Materials. Benzylpenicillin (BP), ampicillin (AMP), and fluoroacetilin (FLU) were a gift from the Commonwealth Serum Laboratories (Parkville, Victoria, Australia); cefotaxime, dicloxacillin, and STZ were purchased from Sigma Chemical Co. (Castle Hill, New South Wales, Australia). Sodium heptanesulfonate was purchased from Biolab Pty (Clayton, Victoria, Australia); acetonitrile (high-performance liquid chromatography [HPLC] grade) was obtained from Mallinkrodt (Clayton, Victoria, Australia), and water was purified and deionized with a Waters Milli-Q unit (Millipore Waters, Brisbane, Queensland, Australia). Male Wistar rats (359 ± 89 g) were used throughout the studies. The animals were housed under standard laboratory conditions (20.0 ± 0.5°C, 55% to 75% humidity, pellet food and water ad libitum).

Methods. (i) HPLC method for analysis of antibiotics. HPLC assays were developed and validated to allow the quantitation of AMP, BP, and FLU in biological samples. A model 250 isotropic pump, LC90 BIO, variable wavelength UV detector with a model 1020 computing integrator (Perkin-Elmer, Glen Waverley, Victoria, Australia) was employed for the assay of all antibiotics. Samples were introduced onto an Altima C18 column (250 by 4.6 mm; inner diameter, 5 μm) (Alltech Assoc., Brisbane, Queensland, Australia) with a Newguard C18 guard column (15 by 2 mm; particle size, 7 μm) (Alltech Assoc.) by using a Shimadzu SIL 9A auto-injector. The mobile phase for analysis of AMP consisted of 10:90 (vol/vol) acetonitrile:0.05 M sodium dihydrogen phosphate (pH 5.0) at a flow rate of 1 ml min⁻¹, with detection effected at 215 nm with cefotaxime as the internal standard. The mobile phase for co-analysis of BP and FLU consisted of 30:70 acetonitrile:0.05 M sodium dihydrogen phosphate (pH 5.0) at a flow rate of 1 ml min⁻¹, with detection effected at 214 nm with dicloxacillin as the internal standard.

Total antibiotic concentrations in urine and plasma were determined by placing an aliquot of plasma (50 μl) or urine (100 μl) into an Eppendorf tube with an equal volume of internal standard solution and then adding acetonitrile to precipitate protein (100 μl to plasma and 200 μl to urine). After vortexing for 30 s, samples were centrifuged at 5,000 × g for 15 min, and the supernatant was removed for analysis.

Tissue samples were weighed into Eppendorf tubes, chopped finely with dissection scissors, and suspended in 200 μl of water. One hundred microliters of the suspension was added to an internal standard (100 μg/ml) was added, and the suspensions were sonicated on ice by using a 3 mm ultrasonic microtip (Sonics and Materials Inc., Danbury, Conn.) for 60 s. Two hundred microliters of acetonitrile was added to precipitate protein and the samples were vortexed for 30 s and then centrifuged at 10,000 × g for 15 min in a bench top centrifuge. The supernatant was then assayed for total antibiotic. Recovery of antibiotic was determined in spiked homogenates at concentrations of 1 to 30 μl ml⁻¹.

(ii) Rat wound model. Rats were rendered diabetic by the injection of STZ (60 mg/kg of body weight intraperitoneally), prepared immediately prior to injection, in sodium citrate buffer (0.15 M NaCl adjusted to pH 4.5 with 0.15 M citrate). Controls received vehicle alone. After 6 weeks, animals with a blood glucose consistently above 350 mg/dl (pre-injection blood glucose, 120.93 ± 13.5 mg/dl), measured with Accutrend glucose test sticks (code 208) and meter (Boehringer Mannheim Australia Pty, Castle Hill, New South Wales, Australia), were classified as diabetic.

Two excisional wounds (15 by 15 mm) were created, either one on each side of the abdomen or one on each thigh, 7 days prior to antibiotic distribution studies and 5 weeks after STZ or vehicle treatment by using previously described methods (7). Briefly, rats were anesthetized with sodium pentobarbitone (60 mg of sodium pentobarbitone/100 g of rat), and hairs surrounding the wound area were removed with electric clippers and Nair depilatory cream before shaving the area with alcoholic chlorhexidine solution and drying the area. Wound areas were marked by using a template, and the area of skin defined by the markings was removed to the level of the subcutaneous fascia by using dissection scissors and then with electric current. The wounds were immediately dressed with Opsite semipermeable transparent self-adhesive dressings, secured at the corners with Supaglue adhesive. Animals were housed singly and for the first 48 h postwounding the dressings were left undis turbed, after which they were removed and the wounds were exposed to the air. Preliminary studies to assess the effect of diabetes on wound contraction rates were performed by monitoring wound areas in a group of six diabetic and four normal rats by repeating daily the tracing of wound sizes with the animals held in a standard crouching position (7). Wound tracings were photocopied (normal size) and computer digitized to give a measure of wound area.

Statistical analysis of the healing rates of the two groups was performed by using analysis of variance to compare mean constants by which each animal’s wound decreased in size. A Kaplan Meir survival analysis statistic was also performed at 90% healing with the log rank test.

Antibiotic distribution studies. On the day of the study, 7 days postwounding, animals were anesthetized with sodium pentobarbitone (60 mg/kg intraperitoneally), and the right jugular vein was cannulated with PE50 tubing (Clay-Adams, Parsippany, N.J.) flushed with heparinized saline. A blood sample was collected to act as a blank for these animals, and the animals left for at least 10 to 15 min to stabilize. After this time filter paper discs soaked in either saline (control) or saline containing freshly prepared 1% ACHs or 1% SNP were placed over the two wound sites and covered with Opsite dressings. After 10 min 50 mg were one of the antibiotics (AMP, BP, and FLU) per kg was introduced together as a single bolus (<0.5 ml) in saline warmed to 37°C into the jugular cannula; this was followed by 0.2 ml of warm saline to flush the cannula. Antibiotics were allowed to circulate for 30 min, after which time a blood sample was withdrawn from the tail vein and the animals were sacrificed by the introduction of 1 ml of concentrated potassium chloride into the jugular cannula. Dressings and filter paper discs were removed from the wounds and discarded, and then wound tissues (granulation tissue, subcutaneous tissue and muscle tissue from below the granulation tissue, surrounding intact skin, and adjacent tissue below the intact skin) were dissected into sterile Eppendorf tubes on ice for HPLC determination of total antibiotic concentrations. The abdomen was then opened and bladder contents were completely aspirated into preweighed Eppendorf tubes on ice for determination of urinary excretion of each of the antibiotics.

RESULTS

HPLC assay. Because of differing retention characteristics, the three antibiotics (AMP, BP, and FLU) could not be analyzed simultaneously by using one isocratic method. Two separate methods were therefore developed with similar mobile phases, one for BP and FLU and one for AMP. Typical chromatograms of the assays are shown in Fig. 1. The retention times of BP, FLU, and the internal standard dicloxacillin were 6.4, 11.3, and 13.9 min, respectively. AMP and its internal standard cefotaxime were eluted at 4.3 and 11.6 min, respectively. Blank injections showed that peaks due to endogenous material were well separated from analytic peaks (data not shown). Detection at 215 nm as opposed to 254 nm allowed greater sensitivity, and quantitation limits of each of the antibiotics in plasma were 0.5, 0.8, and 1.0 μg/ml for AMP, BP, and FLU, respectively. The quantitation limits were 1.0 μg/ml in urine and 2.5 μg/g in tissue for all three antibiotics. Over the concentration range studied, the linearity of response was found to be good for all solutes (r² > 0.994 with negligible intercept), and the mean recovery from tissue homogenates was 87.7% ± 1.6%. The reproducibility of the assays and extractions was good on an inter- and intraday basis (CV[5%] < 7).

Rat wound healing model. Closure rates of the excisional abdominal wounds (15 by 15 mm) in STZ-treated diabetic rats were significantly slower than those in saline-treated nondiabetic controls (analysis of variance, P < 0.005) (Fig. 2). A statistical difference was also shown with the model-indepen dent Kaplan Meir survival analysis at 90% healing (P = 0.006). The wound area-time profiles for both study groups follow an exponential decline, as shown by the straight line semi-log plot of Fig. 2, with retardation of closure in diabetic rats seen more clearly after the first 6 to 7 days.

Antibiotic distribution. The highest plasma concentration at the 30-min sampling point was seen with BP followed by AMP and FLU (Table 1). Although there seemed to be a trend towards lower plasma levels in normal rats, there was no significant difference in plasma concentrations of individual antibiotics between diabetic and normal rats or between animals treated with either of the vasodilators.

Total urinary excretion of individual antibiotics was signifi-
significantly higher in diabetic rats compared with that in nondiabetic controls \((P < 0.05)\). However, the concentrations of antibiotic per milliliter of urine formed (Table 1) were not significantly different for individual antibiotics between diabetic and nondiabetic groups. The highest urine levels for both diabetic and nondiabetic rats were of AMP, which was followed by BP and FLU. The concentrations of AMP, BP, and FLU recovered in the urine represented only a very small fraction of the administered dose, approximately 0.02 and 0.004 for AMP, 0.01 and 0.002 for BP, and 0.005 and 0.001 for FLU in diabetic and nondiabetic groups, respectively. There was no significant difference in the urine concentrations of antibiotics for diabetic and nondiabetic rats between vasodilator treatment groups.

The highest tissue/plasma ratios were seen with AMP followed by FLU and BP (Table 1). Tissue/plasma ratios of antibiotics tended to be higher in abdominal than in peripheral leg wound sites for both diabetic and nondiabetic animals (Fig. 3 and 4). Several of the leg tissues sampled contained no detectable amounts of antibiotic, particularly for BP and FLU, a trend which was much more obvious in ACh-treated animals than in animals that received topical SNP.

The literature shows that AMP has the lowest protein binding capacity of the three antibiotics studied (3) (Table 1). Table 1 shows an estimate of the mean antibiotic tissue concentrations derived from mean tissue/plasma ratios and mean plasma concentrations, together with the approximate mean unbound tissue concentrations calculated by using the published protein binding figures. It can be seen that AMP has 40 and 140 times the unbound tissue concentration of BP and FLU, respectively.

Statistical analysis of tissue/plasma ratios following vasodilator treatment failed to show any level of significance, largely because of the interanimal variability in tissue concentrations. However, results are discussed concerning the general trends seen in the changes in antibiotic tissue/plasma ratios in wound versus contralateral sites and between diabetic and control animals. Treatment of abdominal wound sites with ACh resulted in only a slightly increased level of FLU in granulation tissue over the level of FLU in the contralateral sites and BP in the wound bed of diabetic rats (Fig. 3 and 4). No changes were seen in the tissue/plasma ratios of AMP in abdominal sites in either diabetic or nondiabetic rats (Fig. 3). In the leg wound sites, ACh caused an increase in the tissue/plasma ratio of FLU in wound subcutaneous tissue of diabetic and nondiabetic rats in which low contralateral levels were detected (Fig. 4). No obvious changes were seen in the levels of AMP and BP in either diabetic or nondiabetic rats.

Trends towards higher tissue/plasma ratios of antibiotics following topical treatment of abdominal wounds with SNP, compared with contralateral saline-treated sites, were seen in the granulation and subcutaneous tissue of diabetic rats (Fig. 3). SNP applied topically to abdominal wound sites in nondiabetic rats appeared to have negligible effect on antibiotic concentrations.

In leg wound sites, SNP increased the tissue/plasma ratio of FLU in granulation tissue and the underlying muscle above contralateral levels in diabetic rats, and to a lesser extent in nondiabetic rats (Fig. 4). Variable results were seen in tissue/
plasma ratios of BP; however, increased wound subcutaneous levels were evident following topical SNP treatment compared with contralateral sites. No obvious changes were seen in BP levels in nondiabetic rats (Fig. 4). Concentrations of AMP between SNP-treated and contralateral leg wound sites appeared similar in both diabetic and nondiabetic groups, although the concentrations appeared slightly higher and more variable than those for the ACh-treated group (Fig. 4).

**DISCUSSION**

The antibiotics chosen for the study, AMP, BP, and FLU, are all semisynthetic penicillins which are often prescribed in clinical practice for a variety of soft tissue infections. The HPLC assays and sample extraction procedures developed for AMP, BP, and FLU were shown to be accurate and precise through validation experiments. Both methods utilized simple sample preparation and provided a rapid method for the quantitation of antibiotics in plasma, urine, and tissue.

The rat model of excisional wound healing was based on a previously validated model (7). The induction of diabetes with STZ was chosen after an extensive review of diabetic rat models in the literature. The use of blood glucose above 350 mg/dl to determine diabetes compares well with levels used in the literature of 220 to 360 mg/dl (5, 21). For the present study, it was important that the rat diabetic model chosen should reflect the changes in skin blood flow and peripheral neuropathy which contribute to the delayed wound healing responses in diabetic patients (1, 2, 15, 26, 30, 32, 34, 43). Evidence in the literature points to the suitability of the STZ-treated rat model of diabetes as an experimental model of the human condition also incorporating some of the secondary clinical complications seen in diabetic patients (4, 11, 18, 24, 25, 27, 33, 38, 47).

The significant reduction in wound contraction rates observed in this study can be correlated to impaired wound healing seen in diabetic patients (1, 2, 15, 26, 30, 32, 34). The differences in wound size between diabetic and nondiabetic rats became more pronounced after the first 6 to 7 days, which is consistent with reports of a prolonged inflammatory phase and delayed wound granulation maturation and collagen matrix deposition due to diabetes (10, 16, 17, 31). Statistical difference between the two groups was shown based on our previously published methods (7).

The penetration of systemically administered antibiotics into soft tissue sites is crucial to the success of diabetic ulcer antibiotic therapy. Over recent years it has been highlighted that antibiotic plasma concentrations cannot always be accepted as a reflection of tissue concentrations, particularly at wound sites (36, 41). Contradictory results have been published by Duckworth et al. (9), who found good therapeutic tissue concentrations of clindamycin in four of four diabetic patients treated for foot infections, and Seabrook (36), who found no detectable levels in similar patients using the same dose. It is obvious from these findings that a problem of sufficient antibiotic coverage in tissue sites does exist in many diabetic patients. Variation in the results of previous studies could have been expected, since the authors had little control over the exact tissue sampling points following antibiotic administration prior to surgery, with tissue concentrations presented for individual patients differing in antibiotic administration time by as much as 1 to 4 h by Storm et al. (41), 4.5 to 6.5 h by Duckworth et al. (9), and undefined by Seabrook et al. (36). The present study is effectively a single-dose study and the results are limited to this situation, with further studies being needed to assess the applicability of these findings to chronic antibiotic treatment. Some of the error introduced into previously reported results was eliminated by examining all antibiotic concentrations at a single time point after administration. It is recognized that the distribution phase of each of the chosen antibiotics would differ slightly, and therefore the design of the present study had to enable comparison of the relative distribution between diabetic and nondiabetic wound sites and the effects of vasodilator treatment irrespective of antibiotic time course. This was achieved by the use of sampling periods of exactly 30 min, when the stage of distribution should be similar in each animal, allowing them to act as their own controls.

Observation of the highest tissue/plasma ratios for AMP is consistent with previous studies (36) and reports of a linear relationship between protein binding and the extravascular penetration of antibiotics (39, 46). Differences in urinary excretion of the antibiotics in the study were not expected to have a significant effect on the tissue distribution of the antibiotics between the groups, since the excreted amount represented only a small fraction (0.001 to 0.02) of the administered dose. Studies of the influence of protein binding on the penetration of antibiotics from vasculature into tissue spaces have generated conflicting data. Waterman et al. (44) found no relationship between the protein binding properties of cephaloridine and cefazolin and penetration into polypropylene balls in dogs. Similarly, Gerdin and Hall (14) saw little difference in the penetration of fairly highly bound cephalosporins compared with poorly bound aminoglycosides into peritoneal fluid of rabbits. However, relationships between protein binding and extravascular penetration of antibiotics have been shown with AMP and cloxacinil into human synovial fluid (19) and with β-lactams into human blister fluid (39, 42, 46). The clinical importance of protein binding of antibiotics, however, remains controversial. The protein most often involved in interactions with antibiotics is albumin, and the interactions are generally rapidly reversible and drug molecules constantly undergo binding and release, maintaining an equilibrium between bound and free fractions (6). Consequently, a free fraction of even highly bound antibiotics will be maintained in the plasma by being constantly replaced as the antibiotic enters extravascular spaces. The most important clinical significance of antibiotic protein binding is that the bound fraction does not possess any antimicrobial activity, and thus high tissue concentrations of a highly bound antibiotic may possess only low antimicrobial activity. The results of the present study favor a relationship between unbound concentration and tissue penetration; however, other factors, such as lipophilicity, need to be further addressed to properly predict tissue penetration capabilities.

The most suitable antibiotic for tissue penetration at the time point used in the present study was AMP. Differences in the distribution phases of the antibiotics at the 30 min time

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Plasma (μg/ml)</th>
<th>Tissue (μg/g)</th>
<th>Unbound tissue (μg/g)</th>
<th>Urine (μg/ml)</th>
<th>Tissue/plasma ratioa</th>
<th>Protein binding (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP</td>
<td>30.4 ± 5.9</td>
<td>173.5</td>
<td>142.3</td>
<td>168.2 ± 15.7</td>
<td>5.70 ± 0.5</td>
<td>18</td>
</tr>
<tr>
<td>BP</td>
<td>81.9 ± 16.9</td>
<td>10.7</td>
<td>3.7</td>
<td>64.3 ± 3.8</td>
<td>0.13 ± 0.2</td>
<td>65</td>
</tr>
<tr>
<td>FLU</td>
<td>13.1 ± 3.2</td>
<td>14.8</td>
<td>1.0</td>
<td>37.6 ± 5.0</td>
<td>1.13 ± 0.2</td>
<td>93</td>
</tr>
</tbody>
</table>

* Data (except those for protein binding) are means across all groups (n = 24). Standard errors are indicated for antibiotic distributions in plasma and urine and for tissue/plasma ratios.

* Data are means across all groups and tissues sampled.

Data are from reference 3.

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**TABLE 1. Plasma, tissue, and urine distribution of AMP, BP, and FLU**

The significant reduction in wound contraction rates observed in this study can be correlated to impaired wound healing seen in diabetic patients (1, 2, 15, 26, 30, 32, 34). The differences in wound size between diabetic and nondiabetic groups appeared similar in both diabetic and nondiabetic groups, although the concentrations appeared slightly higher and more variable than those for the ACh-treated group (Fig. 4).

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The most suitable antibiotic for tissue penetration at the time point used in the present study was AMP. Differences in the distribution phases of the antibiotics at the 30 min time
point were not thought to be responsible for the observed results. For example, the half-lives and volumes of distribution of AMP and BP are reputedly similar (1.0 and 0.7 h, and 0.3 and 0.4 liter/kg respectively) (3). The low tissue/plasma ratios of BP but high plasma concentrations of BP suggest that antibiotics with similar physicochemical properties, unless they are extremely potent, would be unsuitable for the systemic treatment of soft tissue infections, particularly as a number of the tissues sampled in the present study showed no detectable concentrations of BP (Fig. 3 and 4). These observations imply that, overall, BP represents a structure with little tissue-pene-

trating power but a reasonable ability to maintain high plasma levels while undergoing moderate excretion into the urine in an unchanged form. The 10 times higher mean tissue/plasma ratios of FLU over BP are confounded by the high protein binding of FLU, meaning that the active unbound fraction in the tissues is much lower than anticipated (Table 1).

The low plasma and urine concentrations of FLU initially suggested that this antibiotic could have the highest fraction of dose distributed into body tissue compartments; however, results from the present study show that in the tissues sampled, this did not appear to be the case. If FLU was partitioning in

FIG. 3. Abdominal tissue/plasma ratios of AMP, BP, and FLU in ACh- or SNP-treated ( ), diabetic; ( ), nondiabetic) and contralateral ( ), diabetic; ( ), nondiabetic) wound sites. Data are means ± standard errors (n = 3).
the tissue spaces, suggested by its low plasma concentration, tissue/plasma ratios would be expected to be much higher. FLU is reported to have a longer half-life (1.5 h) and lower volume of distribution (0.15 liter/kg) than AMP or BP (3), and therefore, would have been expected to be present in the plasma in concentrations higher than those found in the present study. Other possibilities for the distribution of FLU include accumulation in body compartments not sampled or presence in the samples in a metabolized form not detected by our HPLC assay. A further study of the complete time course of FLU distribution and elimination is needed to assess its suitability for tissue penetration, though the present results suggest that FLU may not be the antibiotic of choice for peripheral soft tissue infections, as antibiotic cover does not seem to be maximized in the tissue spaces.

Of the two vasodilators, SNP appeared to have the ability to increase antibiotic tissue/plasma ratios in more of the tissue sites studied compared with contralateral sites than was observed with ACh. It was also noted that for the abdominal wound sites, diabetic animals treated with SNP showed higher

FIG. 4. Leg tissue/plasma ratios of AMP, BP, and FLU in ACh- or SNP-treated (● diabetic; □ nondiabetic) and contralateral (● diabetic; □ nondiabetic) wound sites. Data are means ± standard errors (n = 3).
tissue/plasma ratios for BP and FLU than did normal rats treated with SNP or either group treated with ACh (Fig. 3 and 4). This result could have occurred because of some systemic absorption of SNP over the 30-min treatment period, resulting in increased treated and contralateral levels of the dilator and subsequently the antibiotics (Fig. 3E and F). The direct-acting nature of SNP would be expected to cause an immediate vasodilatation in wound areas where endogenous mechanisms had failed in diabetic animals, whereas in nondiabetic rats it would be expected that vasodilatation in wound sites would already be maximal.

The use of topical vasodilators to promote healing has been attempted in the past. Lishner et al. (22) reported that 20 min of daily soaking of debridged diabetic foot ulcers in a solution of 25% dimethylsulfoxide in saline resulted in increased healing rates over controls. The mechanism behind this response, however, was attributed to a combination of effects, including increased local vasodilatation, decreased thromboxyte aggregation, and increased oxygen diffusion (22). Our review of the current literature has not shown any attempt to increase the local concentration of systemically administered drugs by specific vasodilatation of the microvasculature in or around wound sites. The doses of vasodilator chosen were based on the dose-response studies of Westerman et al. (45), who iontophoresed 1% solutions of ACh (10 s) and sodium nitrite (20 s) through intact skin of diabetics and normal patients and recorded changes in microvascular vasodilatation. It has been demonstrated by our group that iontophoresis effectively delivers solutes through the skin at the same rate seen with passive transport (8, 40). In the present study vasodilators were applied directly to excisional wound sites, with no intact epidermis, and so the doses applied reflected those delivered by iontophoresis by Westerman et al. (45), but with a longer application period to allow for hydration of, and absorption through, surface granulation tissue.

In summary, the present study has shown that the STZ-treated diabetic rat is a suitable model for the study of diabetic-impaired wound healing. The distribution of AMP, BP, and FLU into tissue spaces appears to be related to protein binding, with AMP showing the highest tissue/plasma ratios. The treatment of wound sites with vasodilators to increase local blood flow and antibiotic delivery to the site appears to be more effective with endothelium-independent SNP than with endothelium-dependent ACh in diabetic rats. These results suggest that coadministration of topical vasodilators to wound sites in neuropathic diabetic patients undergoing antibiotic therapy for infected ulcers can increase antibiotic delivery to wound tissue sites.

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REFERENCES


