Dicloxacillin concentrations in amputation

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We treated 18 patients undergoing major lower limb amputation due to ischemia by intravenous administration of 1 g dicloxacillin either 0, 3, or 6 hours before surgery followed by a similar dose 8 hours after the first dose. The administration immediately before the operation resulted in the highest peroperative antibiotic concentrations in the muscular and subcutaneous tissue at the amputation level, exceeding the MIC value of *Staphylococcus aureus*. Similarly, the concentrations in drain fluid were highest in these patients. The median drain secretion later than 12 hours after surgery amounted to only 5 mL, and at that time the concentration of dicloxacillin was less than the MIC of *Staphylococcus aureus* in all the samples.

The risk of wound infection following lower limb amputation is reduced when prophylactic antibiotics are used (1, 2). Prophylactic antibiotics should generally be given closely before the operation (3), but the optimal time of administration when the perfusion is reduced has not been fully established.

We have determined the concentrations of dicloxacillin in serum, subcutaneous and muscular tissue at the level of amputation, and in wound fluid following major lower limb amputation owing to ischemia.

Patients and methods

We studied 18 patients, 12 males and 6 females, with ischemic gangrene, planned for below- or above-the-knee amputation (Table 1). Patients who had received antibacterial therapy within 7 days prior to the amputation were excluded.

The level of amputation was determined after assessment of the skin perfusion pressure according to Holstein and Lassen (4). In three consecutive series the patients received an intravenous bolus injection of 1 g dicloxacillin (Diclocil®, Bristol-Myers) at the beginning of the operation (Group A), and 3 hours (Group B) or 6 hours (Group C) before.

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In all the patients another dose of 1 g was administered 8 hours after the first one.

Before suturing the fascia, specimens from muscle and subcutaneous tissue were taken from the anterolateral edge of the proximal stump. The biopsies were dried clean of adhering superficial blood. Simultaneously, a serum sample was obtained from a cubital vein, and a subfascially located suction drain was inserted. The drain fluid was collected at the following intervals: immediately postoperatively (sample 1), immediately before the injection of the second dose (sample 2), and 12 hours (sample 3) and 36 hours (sample 4) after the injection of the first dose. Then, the drain was removed.

The supernatant fraction was obtained, and all the samples were stored at –70 °C until they were assayed.

The amputation stump was examined 5 and 21 days after surgery.

Antibiotic assay

An agar cup diffusion method was used. Large tryticase agar plates (Statens Seruminstitut, Copenhagen), seeded with *Sarcina lutea* ATCC 9341 as the indicator organism, were employed.

Few colonies of the indicator organism growing on 5 percent blood agar (Statens Seruminstitut) were suspended in 10 mL sterile saline to a density of 0.35–0.40 at 540 nm (model 252 Corning colorimeter). A 1,000-fold dilution of this solution provided an inoculum of approximately 10^6 colony-forming units/mL. Plates were prepared by flooding the plates with 10 mL of this

Table 1. Preoperative patient data (n 18). Median (range)

Group	Age	Weight (kg)	Serum creatinine (mmol/L)	Serum albumin (μmol/L)	Below- /above-the-knee amputation
A	72 (56–89)	58 (40–94)	84 (64–102)	532 (441–616)	5/1
B	72 (40–87)	65 (44–85)	80 (55–110)	493 (410–519)	3/3
C	78 (54–89)	59 (45–90)	103 (77–127)	473 (430–600)	2/4

Table 2. Concentrations (μg/mL) of dicloxacillin in drain fluid immediately postoperatively, 8, 12, and 36 h after the first dose. Median (range)

Group	Postop. (n 13)	8 h (n 12)	12 h (n 17)	36 h (n 13)
A	> 33.3 (> 33.3)	3.4 (2.6–5.8)	2.4 (< 0.4–4.6)	< 0.4 (< 0.4)
B	6.1 (< 0.4–9.2)	0.9 (< 0.4–3.2)	< 0.4 (< 0.4–4.5)	< 0.4 (< 0.4)
C	3.7 (2.1–5.1)	1.1 (< 0.4–2.7)	2.1 (0.5–4.5)	< 0.4 (< 0.4)

standardized suspension and allowed to dry. Wells (10 mm in diameter) were punched in each agar plate.

The tissue biopsies (0.5 g) were homogenized for 1 min in 3 mL phosphate-buffered saline using an Ultra-Turrax® homogenizer. Each homogenate was centrifuged at 15,000 rpm for 5 min, and the supernatant fraction was assayed against a standard prepared from subcutaneous and muscle homogenates, respectively.

Standards and supernatants were pipetted according to a random pattern into the agar wells of the agar plates. After overnight incubation at 35 °C, zones of inhibition were measured with a caliper. The concentrations of dicloxacillin in the supernatant fractions were determined from the semilogarithmic standard curve, and the final concentrations (μg/mL) in the tissue specimens calculated after corrections were made for weight of the biopsies. Correction for blood contamination was not carried out.

Samples of wound fluid and serum were assayed. against standards prepared in pooled wound fluid from 10 patients and pooled human serum (Statens Serum-institut), respectively. Samples and standards were tested only once each. The smallest concentration of antibiotics that could be measured in the tissue was 0.80 μg/mL, and in serum and wound fluid 0.37 μg/mL. Values exceeding 33.3 μg/mL were not further specified.

Statistical methods. The Wilcoxon's paired rank-sum test for data in two groups, the Mann-Whitney test and the Kruskal-Wallis test for unpaired data in two and three groups, respectively, and the Spearman test for rank correlation were used.

Results

Measurable antibiotic tissue concentrations (> 0.80 μg/mL) were only obtained in Group A (Figure 1). Concentrations in muscle were higher than in subcutaneous tissue ($P < 0.05$).

Satisfactory amounts of drain fluid could not be obtained in 17 samples, thus leaving 55 samples to be studied (Table 2). The median volume of the total drain fluid was 20 (8–165) mL; 5 (0–40) mL of this volume was produced later than 12 hours after surgery. No difference in total drain volume was found between patients with above-the-knee (median 40 mL) and below-the-knee amputations (median 20 mL) ($P > 0.50$). In samples 1, 2, and 3 the concentrations of dicloxacillin were higher in Group A than in Groups B and C ($P < 0.05$). For all the patients the median concentration decreased from 6.1 μg/mL in sample 1 to 2.6 μg/mL in sample 2 ($P < 0.01$) The second antibiotic dose did not cause a rise in concentration ($P > 0.60$).

When the samples were taken more than about 200 minutes after administration of the first antibiotic dose, the concentration in the drain fluid was higher than in serum (Figure 1). There was a positive correlation ($\rho = 0.69$, $P < 0.01$) between the concentration in serum and in corresponding drain fluid in sample 1 (Figure 2).

The median operation time was 50 (37–90) min. No cases of wound infection were noticed during the observation period, and none of the patients required a secondary surgical intervention.

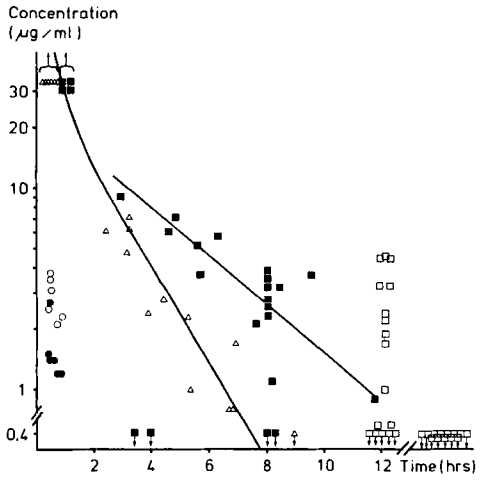

Figure 1. Concentrations of dicloxacillin in serum (△), muscle (○), and subcutaneous tissue (●) plotted against time after the first intravenous injection of 1 g of dicloxacillin, and in drain fluid after the first (■) and second (□) antibiotic dose.
↑ : Plasma concentration levels higher than 33.3 µg/mL.
↓ : Plasma concentration levels lower than 0.37 µg/mL.

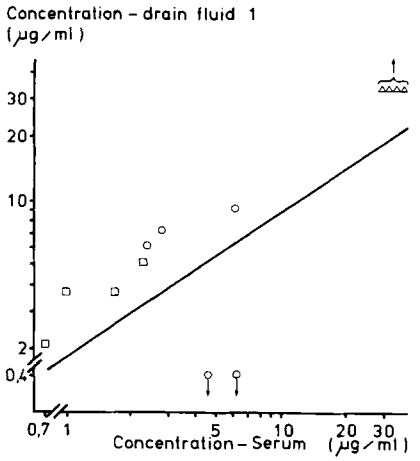

Figure 2. Correlation between concentrations of dicloxacillin in the drain fluid obtained immediately after surgery and peroperative concentrations in serum in all the patients. Group A (△), B (○) and C (□).
↑ : Plasma concentration levels higher than 33.3 µg/mL.
↓ : Plasma concentration levels lower than 0.37 µg/mL.

Discussion

Inadequate extracellular concentrations of an antibiotic like dicloxacillin could be anticipated in tissue with restricted perfusion when the drug is administered at the beginning of the operation. Nevertheless, the highest antibiotic tissue concentrations were obtained when dicloxacillin was administered at this time, as all the tissue values in Group A exceeded the MIC of dicloxcillin for *Staphylococcus aureus* (0.5–0.8 µg/mL) (5), contrary to Groups B and C, in which no measurable antibiotic activity in the tissue could be traced using our methods. Whole-tissue determination underestimates the extracellular concentration by a factor 5 because of extracellular confinement of the drug (6) limiting the value of direct comparison between tissue concentrations and MIC. Efficient tissue levels are obtained shortly after the intravenous administration of this drug because of rapid distribution and equilibrium between the protein-bound and free active fractions of the drug (7, 8). The higher concentrations found in muscle compared with subcutaneous tissue relates to a higher rate of perfusion (9).

Wound fluid is a mixture of wound exudate, blood, and necrotic debris constituting the primary bacterial growth medium (10, 11). The highest peroperative serum concentrations were achieved by intravenous administration at the start of the operation, and ensured sufficient antibiotic concentrations in the wound fluid. The second antibiotic dose did not promote a significant rise in the concentration in drain fluid, indicating that most of the drain fluid is produced at the end of the operation and shortly afterwards.

In agreement with other investigators (12–14), we found that approximately 3 hours after administration, the concentration in drain and wound fluid sustained at a higher level than in serum. The slower rate of elimination in drain fluid could partly be related to a limited exchange of the wound fluid with the systemic circulation.

Retrospective studies (15, 16) indicate that the use of soft latex drains (Penrose) in amputation surgery is associated with an increased risk of wound sepsis. Even when a closed suction drain is applied following total hip replacement there is evidence (17) of migration of skin microorganisms into a wound via the drain or the drain track if the drain is retained for more than 24 hours. Prospective studies are warranted to clarify the benefit of draining the stump after lower limb amputation.

In our study the median drain secretion from 12 hours after surgery until removal of the drain was only 5 mL. In this period the concentration level of dicloxacillin in drain fluid was less than the MIC for *S. aureus* in all the drain samples.

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Acknowledgements

This investigation was supported by Bristol-Myers, Copenhagen, Denmark.