Iontophoresis as a means of delivering antibiotics into allograft bone

R. E. Day, S. Megson, D. Wood

From the Royal Perth Hospital, Perth, Western Australia

Allograft bone is widely used in orthopaedic surgery, but peri-operative infection of the graft remains a common and disastrous complication. The efficacy of systemic prophylactic antibiotics is unproven, and since the graft is avascular it is likely that levels of antibiotic in the graft are low.

Using an electrical potential to accelerate diffusion of antibiotics into allograft bone, high levels were achieved in specimens of both sheep and human allograft. In human bone these ranged from 187.1 mg/kg in endosteal (SD 15.7) to 124.6 (SD 46.2) in periosteal bone for gentamicin and 31.9 (SD 8.9) in endosteal and 2.9 (SD 1.1) in periosteal bone for flucloxacillin. The antibiotics remained active against bacteria in vitro after iontophoresis and continued to elute from the allograft for up to two weeks.

Structural allograft can be supplemented directly with antibiotics using iontophoresis. The technique is simple and inexpensive and offers a potential means of reducing the rate of peri-operative infection in allograft surgery. Iontophoresis into allograft bone may also be applicable to other therapeutic compounds.

The use of allograft bone is well established in limb salvage after tumour surgery and in revision arthroplasty, as morcellised and site-specific structural grafts. Infection is a common complication. The outcome in patients who develop infection is poor and often requires either two-stage revision or amputation.

The risk of peri-operative infection during major allograft surgery has led to the adoption of a protocol of the administration of antibiotics intravenously for 2 to 14 days and orally for up to 16 weeks, although there have been no controlled clinical trials published to show the efficacy of such regimens. The allograft is certainly avascular during this early period, and therefore it is unlikely that therapeutic levels of antibiotics are achieved in the graft using this treatment. To address this issue, numerous attempts have been made to add antibiotics directly to allograft bone, particularly in the field of maxillofacial surgery.

We describe a technique for antibiotic supplementation using iontophoresis, which is a method of accelerating the diffusion of charged ions by applying an external electrical field. Iontophoresis has a long history in medicine, having been used in the 1740s by Pivati to treat arthritis. In their review of the use of iontophoresis in drug delivery, Singh and Maibach describe the enhancement of the transport of drug ions across tissues such as skin, oral mucosa, ocular epithelium and tympanic membrane composed predominantly of lipids and proteins. Iontophoresis was used in our study to transfer ionised antibiotics into the cortex of allografts, with the cortical bone acting as the membrane. The techniques which we describe can be applied pre-operatively in the theatre to allografts before implantation. The antibiotic-loaded allograft can then act as a drug depot in vivo, diffusing antibiotics into the periosteal tissues to prevent peri-operative bacterial colonisation of the surface of the allograft. A preliminary study using flat sections of bone established that iontophoresis could move ions into cortical bone, with a pulsed field offering no advantage over constant voltage, and that the best direction for ion transport was across the thickness of the cortex. From this work a clinical model was developed which is now described as are the bio-availability and bioactivity of the supplemented graft.

Materials and Methods

Experimental work was carried out using mature Merino sheep tibiae and human allograft bone which had been rejected for implantation because of missing serology by the Perth
imens were prepared in an identical fashion, but no iontophoresis was required. Active samples were iontophoresed at 90 V using Methylene Blue (Methylene Blue Injection USP; DBL, Perth, Australia) for 1, 2, 5 or 10 minutes. After washing and drying, transverse sections were cut using a low-speed diamond saw (Buehler IsoMet; Buehler Ltd., Lake Bluff, Illinois) and the penetration of the Methylene Blue was measured macroscopically. In addition, histological sections 300 µm thick were prepared to assess the penetration at microscopic level.

**Quantitative analysis.** Sections of sheep tibia were iontophoresed at 90 V with 1% solutions of either gentamicin sulphate (Gentamicin Injection BP; Pharmacia & Upjohn, Sydney, Australia) or flucloxacillin sodium (Flucloxacillin Sodium for Injection; DBL, Perth, Australia) in de-ionised water. Iontophoresis was carried out for 1, 5 or 10 minutes (n = 5 at each time point) with controls (n = 5) at 0 V for ten minutes. After washing and drying, samples were taken from both the endosteal and periosteal surfaces by drilling the bone using a 3 mm low-speed drill in ten positions on each surface and then crushed to a fine powder. We added 2 ml of distilled water to a measured mass of each powdered sample and agitated them using ultrasound. After centrifuging, the supernatant fluid was removed and the process repeated twice more, the last wash being left to soak for 12 hours before centrifuging. The flucloxacillin specimens were refrigerated to 4°C during this period to minimise degradation. The supernatant solutions were analysed for antibiotic content using fluorescence polarisation immunoassay (Abbott Axsym Analyser; Abbott Laboratories, Abbott Park, Illinois) for gentamicin and high-performance liquid chromatography for flucloxacillin. From the levels in the supernatant solutions, the concentrations of antibiotic in the bone samples were calculated and expressed as the mean plus or minus 1 SD.

This process was repeated using two non-irradiated human tibiae which were cut into sections 20 mm long. Because of the scarcity of suitable bone, only one section was used for each time point.

The concentrations of antibiotic for the repeated trials in sheep bone were log transformed to stabilise their variance, since investigation of the assay system had shown that variance was proportional to the concentration. One-way analysis of variance (ANOVA) was performed and, if this was significant, Tukey’s test for multiple comparison of means was used to determine the effect of iontophoresis time on concentration.

**Bio-availability.** Antibiotic bio-availability was assessed using five specimens of sheep tibia iontophoresed as above at 90 V for five minutes. Samples were taken from both cortices as above for assay of the starting antibiotic concentration. Each bone specimen was incubated in 20 ml of Ringer’s solution. This solution was changed at intervals, up to 14 days, and the level of antibiotic in the solution assayed to determine the rate of elution of the iontophore-
sed antibiotic. Data were expressed as the percentage of the original antibiotic present in the bone.

**Bio-activity.** Sheep tibial specimens were prepared as above in five groups as follows: no exposure to antibiotics (control), one soaked for five minutes in each antibiotic solution and one iontophoresed at 90 V for five minutes using each antibiotic. From each specimen a transverse section 1 mm thick was cut using the diamond saw, rinsed with water and placed in the centre of a nutrient agar plate flooded with a heavy suspension (10^10 orgs/l) of *Staphylococcus aureus NCTC 6571*, which was found to have a minimum inhibitory concentration of 0.064 mg/l for gentamicin. The plates were incubated for 24 hours at 35˚C and the zone of inhibition inspected and measured macroscopically with a caliper.

**Thermal effects.** To determine the heating effect of iontophoresis on bone, three sheep tibial samples were prepared as above with a fibre-optic thermal fluorescence probe (Multichannel Fluoroptic Thermometer Model 2000; Luxtron, Santa Clara, California) placed in a drill hole 6 mm deep and 0.8 mm in diameter in the middle of the cortex. The apparatus was otherwise set up as above and then cooled to 10˚C to simulate an initially frozen-allograft. It was operated at 90 V for 15 minutes and a continuous recording of the temperature made.

**Bidirectional iontophoresis.** Using the same apparatus, five sections of human cortical bone were iontophoresed at 90 V for five minutes with 1% gentamicin solution in the medullary canal and 1% flucloxacillin solution in the beaker. This produced a flow of gentamicin from the endosteal to the periosteal surface and of flucloxacillin from the periosteal to the endosteal surface of the bone. Samples were taken and crushed for quantitative analysis as above. One-way ANOVA was used to test for significant differences between the control specimens and those taken at five minutes for both periosteal and endosteal surfaces.

**Results**

**Qualitative evaluation.** With this apparatus and a potential difference of 90 V, typical cell currents of around 40 mA were found in sections of sheep tibia 20 mm long and iontophoresed using Methylene Blue. Colouration of the periosteal surface was noted by approximately 1.5 minutes and was maximal by 5 minutes. Macroscopic evaluation of the specimens showed penetration of the dye increasing with time and microscopic evaluation showed uniform staining of the penetrated region of the bone.

The results for human tibial bone were comparable with those of the sheep model. The human tibiae had thicker cortices and, unlike sheep tibiae, had some cancellous bone on the endosteal aspect. This cancellous bone was no barrier to diffusion of the dye, and the rate and uniformity of penetration were comparable in both human and sheep bone, although full penetration occurred later in the thicker human bone.

**Quantitative evaluation.** The levels of gentamicin in the sheep tibiae reached a plateau of about 150 mg/kg of bone on both the periosteal and endosteal surfaces (Fig. 2a). The peak in the endosteal samples was reached by one minute and in the periosteal samples at five minutes so that after eight minutes a plateau had been achieved in both the endosteal and periosteal surfaces. The level of flucloxacillin in the endosteal samples peaked at one minute at between 60 and 80 mg/kg, but the periosteal samples only achieved levels of 10 to 15 mg/kg even after five minutes (Fig. 2b).

ANOVA showed a significant effect (p < 0.01) for all conditions except flucloxacillin on the periosteal surface (p = 0.057). Tukey’s test was applied only when ANOVA indi-
icated a significant effect of iontophoresis, and showed that the 5- and 10-minute results were the same for gentamicin on the periosteal surface while the 1-, 5- and 10-minute results were the same for both antibiotics on the endosteal surface. These results confirm the visual impression of the plotted data.

In the human tibia, the endosteal levels of gentamicin achieved were similar to those obtained in sheep tibiae but the rate of penetration of antibiotic was slower. Endosteal levels of gentamicin increased progressively with time and reached a plateau of about 250 mg/kg of bone at ten minutes. The periosteal levels were about 250 mg/kg at ten
minutes at which time they had not reached a plateau (Fig. 3a). Both periosteal and endosteal levels of flucloxacillin increased throughout the period of iontophoresis rather than reaching a plateau and achieved levels of 65 mg/kg and 242 mg/kg of bone, respectively, after ten minutes (Fig. 3b).

Bio-activity. Figures 4a to 4e show the inhibition of growth of Staph. aureus by sections of bone which had different exposures to gentamicin or flucloxacillin. While bacterial colonies grew up to the bone in the control specimen which was not exposed to antibiotics, there was a zone of inhibition around the specimens which had been soaked in antibiotic solutions for five minutes and a larger zone of inhibition around those which had been treated using iontophoresis for the same time.

Bio-availability. The results for the rates of elution of the antibiotics from tubular sections of bone are shown in Figure 5. Both antibiotics behaved in a similar fashion, with a logarithmic decrease in their rates of elution. Antibiotic continued to be eluted for up to two weeks into Ringer’s solution.

Thermal effects. After an initial rapid rise in the first 30 seconds, the temperature in the bone cortex increased slowly to a maximum of 35.7˚C at 15 minutes after which the iontophoresis was stopped. The mean current was 40 mA at 90 V, giving a total power dissipation in the iontophoresis cell of 3.6 W.

Bidirectional iontophoresis. The levels of gentamicin and flucloxacillin measured after bidirectional iontophoresis for five minutes are shown in Table II. The mean level of gentamicin achieved was 187.1 mg/kg of bone (SD 15.7, p < 0.001) on the endosteal surface where the gentamicin originated and 124.6 mg/kg of bone (SD 46.2, p = 0.018) on the periosteal surface. The levels of flucloxacillin were much lower at 31.9 mg/kg of bone (SD 8.9, p = 0.011) on the periosteal and 2.9 mg/kg of bone (SD 1.1, p = 0.021) on the endosteal surface. All of these results were significantly different from the control findings.

Discussion

Structural bone allografts have provided a solution to many reconstructive problems in musculoskeletal and maxillofacial surgery, but infection of the graft remains a significant complication. While the use of small frozen-allografts has a low rate of infection,21 major allografts have rates of infection of between 5% and 18%.3-5,13-15,17,21 These high rates are not surprising because allografts are avascular and allograft surgery often involves extensive soft-tissue excision, large wounds, long operating times and the potential for wound breakdown.13,21 Concurrent chemotherapy or radiotherapy also causes immunosuppression and can lead to failure of incorporation of the graft.

Allograft infections generally present early, with approximately 75% occurring within four months.13,15,21,22 Perioperative infection is the most likely cause of most of these infections. The most common organisms isolated are
Gram-positive (54%), followed by Gram-negative (36%) and mixed (10%). Prophylactic antibiotic regimens suggested in the literature involve the administration of antibiotics intravenously for 2 to 14 days and orally for up to 16 weeks. However, even these rigorous regimens are unlikely to generate high levels of antibiotic in the avascular allograft.

There have been several attempts to supplement allografts with antibiotics by soaking morcellised cancellous bone graft in antibiotic solutions. In an effort to achieve a more sustained release of antibiotics, demineralised allograft mixed with antibiotic powders has been tried and, with the addition of gelatin, has been shown to reduce infection in a dog model and clinically in infected molar roots. Compared with milled cancellous graft, however, cortical grafts are very dense, with a small surface area and large volume, making them unsuitable candidates for the introduction of antibiotics through a soaking regimen.

This is the first published report of the use of iontophoresis to introduce therapeutic agents directly into allograft bone. Using this procedure, high concentrations of both gentamicin and flucloxacillin were achieved in sheep tibiae after one minute and in human allograft tibia after five minutes. The rate of transport of flucloxacillin was lower than that of gentamicin, perhaps because flucloxacillin forms ionic aggregates in solution which are larger than the unaggregated molecule and thus are iontophoresed at slower rates. This effect was seen in both sheep and human bone, but did not prevent flucloxacillin reaching therapeutic levels on the adjacent cortical surface. As with any diffusion process, the adjacent surface of the bone received more of the antibiotic than the far surface.

Because the basic process is still diffusion, the actual length of time required for complete penetration of a bone varies with the thickness of the cortices and perhaps the density of the bone. For the relatively thin cortices of sheep tibiae, a steady state was reached after five minutes. The slightly thicker human tibia had adequate levels at five minutes, but no plateau had been reached and the trend was still upwards. Thicker bones, such as femoral shafts, require longer periods. The point at which a steady plateau is reached is of course the optimum length of time for iontophoresis.

There is a maximum useful time for iontophoresis, since if there is an inadequate reservoir of antibiotic, it is possible to drive all of the available ions through the bone, reducing the amount retained in the bone. The exact relation between bone geometry, current and time is not clear, but preliminary results indicate that iontophoresis for over 30 minutes can be sustained before depletion occurs with this apparatus. Accordingly, a conservative approach suggests that iontophoresis for about 20 minutes will give maximum antibiotic loading while ensuring that depletion does not occur. Since antibiotic ion transfer is related to the current flow in the cell, monitoring the current during iontophoresis may allow the process for each bone to be optimised.

Simultaneous transport of gentamicin and flucloxacillin was demonstrated, the normally incompatible drugs being separated by the impermeable bone. With gentamicin added to the endosteal aspect and flucloxacillin to the periosteal aspect, gentamicin penetrated the full thickness, but flucloxacillin cover of the endosteal surface was poor, probably as a consequence of the aggregation process.

Intravenous administration of antibiotics has been reported to produce levels in bone of 2.4 to 19.4 mg/kg. In contrast to these modest levels, iontophoretic treatment introduced up to 250 mg of gentamicin and 80 mg of flucloxacillin per kilogram of bone in the allograft. These may seem high levels of antibiotic, but an allograft of 500 g loaded with 250 mg/kg of bone would only contain a total dose of 125 mg of gentamicin, about half of the commonly used daily dose of 240 mg.

The use of local antibiotic therapy for prophylaxis and treatment of infection in orthopaedic practice is well established and much work has been done on the use of polymethylmethacrylate beads and antibiotic-loaded cement in the clinical setting. The aim of local treatment is to achieve high concentrations of the therapeutic agent at the site of the pathology, while avoiding the possible complications of high systemic levels. Iontophoretically-loaded allograft bone can achieve this desirable situation. Furthermore, because the antibiotics diffuse out from all surfaces of the bone, the concentration of antibiotics is highest at the surface of the bone, even inside small holes and cracks. This contrasts with the situation when supplementary antibiotics are introduced into the wound, whether from the bloodstream or by antibiotic-loaded cement, since the concentration gradient is in the opposite direction. By supplementing the bone directly, iontophoresis should ensure antibiotic cover even in the smallest crevices and cracks on the surface where bacteria would normally be protected from the body’s defences.

We have demonstrated that ions can move through bone under the influence of an externally-applied potential difference, that therapeutic levels of antibiotics can be achieved in bone by iontophoresis and that these antibiotics remain bio-available and bio-active. The procedure is simple and inexpensive and provides a potential adjunct to major allograft surgery. Although some extra handling of the allograft is required to seal the nutrient foramina, this only adds about five minutes to the normal preparation time. The iontophoresis can be done on a separate trolley in the theatre in parallel to the surgery, reducing the additional time required. The apparatus is being developed to reduce the complexity of the process and the clinical efficacy of local antibiotic therapy delivered this way is currently being tested.

The author or one or more of the authors have received or will receive benefits for personal or professional use from a commercial party related directly or indirectly to the subject of this article. In addition, benefits have been or will be directed to a research fund, foundation, educational institution, or other non-profit organisation with which one or more of the authors are associated.
References


