Evaluation of the viability of bone fragments

AN EXPERIMENTAL STUDY USING LASER DOPPLER FLOWMETRY

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We used laser Doppler flowmetry (LDF) to measure flux in cortical bone fragments as a method of determining their vascular status and viability. In an experimental tibial osteotomy, measurements of flux were made from specific cortical sites both before and after osteotomy. Flux levels fell rapidly in non-vascularised fragments and remained significantly reduced throughout the experiment. By contrast, those in vascularised fragments were significantly reduced one and two hours after the osteotomy but then increased. From three hours after the osteotomy, there was no significant difference in flux levels between the vascularised fragments and proximal bone stock.

We conclude that measurement of bone flux by LDF may have a role in the objective evaluation of the viability of bone fragments, but that further studies are required to validate the technique before its adoption in the management of the injured patient.

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Long bones which are intact have a dual blood supply involving both endosteal and periosteal vessels. Fracture often leads to the production of bone fragments with a disrupted endosteal blood supply. These fragments become dependent upon the periosteal blood supply for maintenance of their vascularity and hence viability. The initial treatment of open fractures requires debridement, with removal of non-viable tissue. Inadequate debridement predisposes to infection, both in soft tissues and bone, which prejudices the repair of fractures. There are well-established criteria for the intraoperative determination of muscle viability, but not at present for bone. Those fragments with a reasonable soft-tissue attachment are believed to maintain a blood supply which is sufficient to ensure viability.

Many techniques have been used for determining blood flow to bone. These include MRI, radioactive tracer techniques (including the use of microspheres), laser Doppler flowmetry (LDF), measurement of the oxygen tension and microangiography. The use of some of these, e.g. microangiography, is restricted by the need to remove tissue for evaluation. Others, e.g. scintigraphy, allow only one assessment to be made during a finite period.

LDF may be an appropriate technique for evaluating blood flow to bone in vivo, and provide a scientific basis for debridement of fracture wounds. The technique has been used for measuring flux in a number of tissues, including bone and tooth. It has also been employed to monitor the progress of the healing of fractures in experimental animals and as an adjunct in the surgical management of osteomyelitis. It has not been used, however, to determine blood flow in bone fragments from fractures. We have therefore evaluated the use of LDF in determining changes in blood flow in bone fragments in the immediate period after osteotomy.

Materials and Methods

Animal model. Six adult female sheep (mean weight ± SEM, 77 ± 4 kg) were anaesthetised by the inhalation of oxygen/nitrous oxide and halothane. Anaesthesia was maintained by the continuous intravenous infusion of ketamine and midazolam administered via a catheter in the jugular vein. Fluids (0.18% glucose-saline solution) were given intravenously at a rate of 2.5 ml kg⁻¹ hr⁻¹. The common carotid artery was cannulated and the arterial blood pressure measured with a Propaq EL106 monitor (Protocol Systems Inc, Beaverton, Oregon).

The medial cortex of both tibiae was exposed, and six sites (each 2 cm apart) were marked on the surface using a template. At each site, a small incision (2 × 2 mm) was made in the periosteum to allow placement of the LDF probe on to the cortex. Baseline readings were taken at each position along the intact bone (Fig. 1). Data were acquired over a 20-second period. The mean values were calculated from the flux readings taken at the two meas-
A cortical osteotomy was made in the mid-shaft of each tibia with an orthopaedic saw, creating a full-thickness cortical fragment (approximate dimensions $3 \times 1$ cm). In the left tibia all periosteal attachments to the fragment were divided, rendering it avascular. On the right side the periosteal attachment along one edge of the fragment was preserved, thereby retaining a periosteal blood supply. After osteotomy, flux readings were recorded at all sites, every hour for six hours, and then every two hours, until 12 hours after osteotomy. The arterial blood pressure was recorded continuously. After 12 hours, both external iliac arteries were cannulated and an angiogram was performed using barium sulphate solution (Polibar Rapid; E-Z-EM Ltd, London, UK) infused at a pressure of 350 mmHg after the intravenous administration of 5000 IU of heparin. The animals were killed by the injection of sodium pentobarbitone. Radiography was performed on both hind legs and the presence or absence of barium in the fragments was determined (Figs 2 and 3).

**Results**

**Blood pressure.** During the 12 hours of monitoring, the mean arterial pressure fell from 109.8 ± 4.4 mmHg to 86.1 ± 8 mmHg (Fig. 4).

**Flux readings**

**Baseline levels (Time 0).** There was no significant difference in baseline flux levels between comparable sites on the two tibiae, and no significant difference in baseline flux at the six measurement sites along each (Table I).

**After osteotomy.** In all fragments, changes in flux were compared with baseline levels recorded from the predetermined positions before the creation of the fragment, and with the flux levels recorded from viable bone of the proximal tibia and from the distal tibia.

**Detached fragments.** Thirty minutes after the osteotomy the detached fragments showed a significant reduction in flux with respect to baseline values which remained for the duration of the experiment (Fig. 5). At all time points after 30 minutes post-osteotomy, flux readings in the detached fragments were significantly lower than those in both the proximal and the distal bone.

**Attached fragments.** These showed an initial reduction in flux compared with baseline values which reached statistical significance at one hour ($p = 0.002$). By three hours, this reduction was not statistically significant (Fig. 6). When flux readings at the attached fragment were compared with those at the proximal tibia, the values at one and two hours were significantly reduced ($p = 0.013$ and $p = 0.002$, respectively).
At all other times there was no significant difference in flux values recorded from the attached fragments compared with the proximal tibia. Flux readings at the attached fragment were significantly lower than those in the distal tibia for up to six hours after the osteotomy (p < 0.05).

The flux readings from both attached and detached fragments fell after osteotomy and the reduction was greatest in detached fragments. There was a significant difference in flux readings between the fragments at 30 minutes (p = 0.0002) and after three hours (p = 0.0008).

Repeated measurements over time showed that for flux readings from the proximal bone stock there was an overall reduction in flux with time, but there was no significant difference between the two groups.

Discussion

LDF has many potential advantages for measuring the blood flow of bones in vivo. It is a direct method which does not require the removal of tissue as part of the investigation and allows the serial evaluation of the blood flow. There are several reports of its use in vitro and under these circumstances the reproducibility is high.

The Moor DRT4 system used in this study produces an output in arbitrary flux units by contrast to other systems which produce an output in millivolts. Some authors have quoted absolute values in millivolts as being a reliable discriminator between viable and non-viable bone.

When using the laser probe, readings fluctuated for up to one minute when initially recording from a new site. In addition, movement of the cable caused a fluctuation in readings. Movement artefacts have been reported by other authors. In this study it was necessary to ensure an appropriate delay between placing a probe at a new site and beginning data capture. Furthermore, to reduce any possible bias, the operator holding the probe was not able to see the output screen during the period of examination. Construction of a small periosteal window to expose the cortex may, theoretically, cause disruption of small periosteal-osseous vessels, and disturb the flux in the fragment. Preliminary studies have shown this to be unlikely since similar flux readings were obtained by making readings from bone either with the periosteum intact, or after creation of a small periosteal window. Measurements of flux

Table I. Mean ± sd baseline flux (afu) at left and right tibiae (n = 6)

<table>
<thead>
<tr>
<th>Site</th>
<th>Left leg</th>
<th>Right leg</th>
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<tbody>
<tr>
<td>1</td>
<td>50.2 ± 26.2</td>
<td>77.1 ± 50.9</td>
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<tr>
<td>2</td>
<td>53.3 ± 24.1</td>
<td>54.9 ± 17.7</td>
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<tr>
<td>3</td>
<td>49.2 ± 13.7</td>
<td>51.0 ± 12.4</td>
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<tr>
<td>4</td>
<td>66.0 ± 19.8</td>
<td>48.1 ± 19.9</td>
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<tr>
<td>5</td>
<td>83.7 ± 46.3</td>
<td>58.9 ± 27.4</td>
</tr>
<tr>
<td>6</td>
<td>58.2 ± 29.1</td>
<td>73.1 ± 17.4</td>
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made through the intact periosteum, however, can lead to variable results, dependent on the pressure applied with the probe; it was for this reason that measurements were made directly from the cortical surface after the creation of the periosteal window.

Baseline values obtained from the tibiae, before osteotomy, showed a relatively large standard deviation (Table I). This finding provides support for the use of LDF as a comparative measurement technique (using a reference point from apparently normal bone in the animal under study) rather than relying on absolute values of flux.

Immediately after osteotomy there was a reduction in flux at both attached and detached fragments. This is similar to findings reported with the use of LDF at osteotomy sites. Swiontkowski et al. showed a reduction in flux of 60% at the femoral head after osteotomy in pigs, and Hupel, Askenov and Schemitsch a reduction of 50% after a mid-shaft tibial osteotomy in dogs.

The behaviour of the detached fragments was as expected, in view of their complete devascularisation, with a rapid reduction in flux. This was followed by maintenance of a low level of flux throughout the 12 hours, but it did not reach zero at any time. Similar findings of a failure of flux levels to fall to zero, despite cessation of regional blood flow, have been reported in other tissues. The changes in flux in the attached fragments were more complex, with an initial reduction at one hour followed by an increase by three hours after osteotomy. It was only between one and two hours after osteotomy that there was a significant difference in the levels of flux of these fragments with respect to the proximal bone stock. By comparison, there was a significant difference between levels of flux in vascularised fragments and the distal bone up to six hours after osteotomy. These results would indicate that the proximal bone is the better reference point for LDF measurements.

The determination of the difference in flux readings between viable bone and fragments is an important concept when considering the potential clinical application of LDF to the management of fractures. There was a significant difference for these values for detached (non-vascularised) fragments from 30 minutes, whereas for attached (vascularised) fragments there was no significant difference, with respect to the proximal bone, from three hours.

The time at which recordings of flux are made appears to be critical to the interpretation of the results. For fragments with a periosteal attachment, the results of LDF obtained soon after osteotomy may give a result which suggests that the fragment is non-viable, despite this probably not being the case.

In conclusion, our findings indicate that LDF may have a role in distinguishing between vascularised and avascular bone fragments, but the timing of the measurement would appear to be critical. LDF may help in the intraoperative evaluation of bone fragments at a fracture, although the pattern of periosteal attachment will be more variable than that seen in this experimental model. There is a need therefore for further experimental studies of the use of LDF to be undertaken before it can be used in the management of the injured patient.

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References