Depression of bone blood flow after blunt trauma

A fracture study in the adult rabbit

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Delayed union of long bone fractures is commonly ascribed to deficient vascularity, but bone blood flow after fractures caused by blunt trauma has yet to be quantified. We have studied blood flow to the tibial diaphysis after such fractures, and compared the results with those found after osteotomy. 24 adult New Zealand White rabbits were studied. Fractures of the tibial shaft were produced under anesthesia by percussion and immobilized in a cast. Blood flow to the tibial diaphysis was measured at 1 and 2 weeks using the microsphere method. Cortical blood flow proximal to the fracture was increased at both time intervals, and distally at 2 weeks. Marrow flow was depressed distally at 1 week. Marrow flows at 1 week and distal cortical flows at 2 weeks were reduced in comparison with osteotomies studied previously. This depression of blood flow response by blunt trauma prompts further investigation of the role of vascular factors in delayed union.

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The great majority of investigations into the blood supply of bone after fracture have been carried out on surgical osteotomies. These may bear little resemblance to fractures caused by blunt trauma, particularly with regard to the associated soft tissue injury. We have studied blood flow response to fracture of the rabbit tibia caused by blunt trauma.

Method

The study was performed on a total of 24 adult female New Zealand White rabbits weighing 3.4–4.5 kg. The rig used to produce tibial shaft fractures was a modification of that used by Edwards (1965). Into a heavy base plate were set 2 parallel vertical bars, along which ran a weight bearing a rounded point. To allow a fracture by three-point bending, the right tibia was held under the weight by supports around the hock and knee.

The animals were anesthetized with Hypnorm (Janssen, Oxford, UK) and halothane. Analgesia was supplemented by percutaneous infiltration of 1 mL of 2 percent plain lidocaine behind the tibia at its midpoint, the site of fracture. The rotation of the limb was adjusted until the anteromedial crest of the tibia was uppermost, to allow the limb to be struck without the imposition of muscle between the weight and the bone. This reduced variability between the fractures of different animals. The skin was protected by a double layer of cast padding (Soffban, Smith and Nephew, Hull, UK). The weight was raised 40 cm above the point where it rested on the limb and was allowed to fall onto the limb under gravity. In twelve animals a weight of 0.96 kg was used, and in the remainder the weight was doubled to 1.91 kg.

The rig was successful in producing fractures with minimal skin problems, although approximately one fifth of fractures were segmental, presumably as a result of angulatory forces at the limits of the limb supports. In some cases more than one attempt was required to produce a fracture, but the number of animals involved was too small to allow statistical analysis of any differences between these and the remainder.

After fracture, manual traction was applied to the limb while it was placed in a padded cast (Triffitt et al. 1992). Lateral radiographs of the fracture were obtained within the cast. Postoperative analgesia was supplemented by intramuscular buprenorphine. On the second or third day the animals were returned to the free-range room, although they had not regained full mobility at the time of blood flow measurement.

5 animals were excluded from the analysis as the fractures produced were segmental, 4 from the 1-week group and 1 from the 2-week group, and a further case was excluded from the 1-week group after the occurrence of skin necrosis over the fracture site (Edwards

1965). There were no significant differences after the use of the 2 different weights, and the results were combined for the 2 time periods. In 97 percent of samples the number of microspheres exceeded 150, the number above which a further increase does not improve the accuracy of the method in bone (Li et al. 1989).

In most cases the skin was punctured from within out as a result of tenting of the skin at the fracture site during the displacement of the fracture. The wounds were apparent only because of the formation of a drop of blood on the skin surface. At the time of blood flow measurement, the skin was intact in all but the one case excluded after the development of skin necrosis. Except for 2 cases in the 1-week group and 1 in the 2-week group, the fractures displaced while the limb was in the cast, as observed after osteotomy (Triffitt et al. 1993), resulting in shortening of up to 12 mm.

Blood flow measurement

12 animals were studied 1 week after fracture, and the remaining 12 after 2 weeks. Measurement of blood flow to the tibial diaphysis and skeletal muscle was performed as previously described (Triffitt and Gregg 1990) using 4.8–5.0 × 10^6 microspheres sized 11.3 ± 0.1 µm and labeled with ^{113}Sn. Briefly, the microsphere injectate was delivered into the aortic root and a reference sample withdrawn over a period of 2 min from a brachial or carotid artery. The tibias were removed, together with a sample of skeletal muscle from the anterior compartment. The metaphyses were discarded and the diaphysis was divided into proximal and distal fragments, this division being formed by the fracture in the experimental bone, and by a saw cut at the equivalent level in the control bone (Triffitt et al. 1993). Additional cortical fragments arising from any fracture comminution were included in the sample of the parent fragment, and the marrow removed manually into separate specimens. The cortical bone was dissolved in acid and these and the reference samples centrifuged before isotope counts were obtained. Correction of the isotope counts was made for the varying heights of the marrow and muscle samples. Flow was calculated from the formula:

$$F_s = (C_s / C_{ref})\, F_{ref}$$

where F_s specimen flow, C_s specimen count, C_{ref} reference sample count, and F_{ref} reference sample flow. The number of microspheres in each sample was calculated by dividing C_s by the radioactivity of each sphere, corrected for decay (Warren and Ledingham 1974).

Analysis

Flows to the 2 limbs were compared, using the paired *t*-test. Comparisons between the 1- and 2-week groups were made by comparing the ratios of the flows to the experimental and control limbs. The null hypothesis is that the ratio of the ratios from 2 different groups is unity. This hypothesis was therefore tested by applying the unpaired *t*-test to the logarithms of the ratios. The same method was applied to compare the results of the present study with those of cast immobilization alone and of surgical osteotomy, as investigated previously (Triffitt et al. 1992, 1993). The results obtained with the 2 fracture energies were not significantly different and were considered together.

Results

Cortical flow was found to increase with respect to the control limb, proximally at both 1 and 2 weeks and distally at 2 weeks (Table 1). Marrow flow was depressed distally at 1 week. Muscle flow had increased by 2 weeks. Comparing the 1- and 2-week groups, flow was higher at 2 weeks than at 1 week in the distal cortex and in both proximal and distal marrow.

Compared to cast immobilization alone (Triffitt et al. 1992) marrow flows were depressed at 1 week, and cortical flow raised proximally at 1 week and both proximally and distally at 2 weeks (Table 2). On comparison with surgical osteotomy (Triffitt et al. 1993), marrow flows were depressed at 1 week, and the rise in distal cortical flow at 2 weeks was reduced.

Discussion

There was considerable variability between animals in the estimates of blood flow. There are limited comparable data from microsphere fracture studies with which to compare the standard errors in the data, and none from studies of blunt trauma. Our errors are smaller than those of Chidgey et al. (1986) and similar to those of Smith et al. (1990), Strachan et al. (1990), Keller et al. (1991), Wallace et al. (1991), and our own figures after osteotomy (Triffitt et al. 1993). The degree of variability emphasizes the importance of reference to a control limb.

The comparative reduction in marrow flow at 1 week, and the absence of a rise to parallel that in the cortex at 2 weeks, supports the conclusion from exclusion experiments (Triffitt et al. 1993) that the cortical flow response is mediated by a supply parallel to that to the marrow, probably that from the periosteum and muscle. The mechanism of the changes in marrow

Table 1. Blood flow (mL/min 100g) after a unilateral tibial diaphyseal fracture. Mean ± *SE*

		Control		Fracture		*P*-values[a]
Proximal cortex	1[b]	1.07	*0.41*	1.71	*0.55*	<0.002
	2[c]	0.72	*0.14*	1.64	*0.32*	<0.01
Distal cortex	1	2.10	*0.61*	3.02	*0.88*	NS
	2	2.10	*0.38*	4.91	*1.12*	<0.01
Proximal marrow	1	29.45	*10.20*	16.35	*5.06*	NS
	2	29.21	*4.62*	29.45	*4.97*	NS
Distal marrow	1	11.87	*3.25*	5.26	*1.43*	<0.02
	2	9.39	*1.20*	9.95	*1.89*	NS
Skeletal muscle	1	3.00	*0.54*	7.21	*2.59*	NS
	2	2.88	*0.43*	5.24	*1.12*	<0.05

[a] Values refer to differences between the limbs (paired *t*-test)
[b] 1-week group, n 7
[c] 2-week group, n 11

Table 2. Ratios of flows to experimental and control limbs. Ratios are given as geometric means, and are compared by the unpaired *t*-test

	One week		Two weeks	
	Ratio	*P*	Ratio	*P*
Proximal cortex				
cast[a]	0.81		0.95	
fracture[b]	1.79	<0.001	2.22	<0.001
osteotomy[c]	2.05	NS[d]	3.17	NS
Distal cortex				
cast	1.30		1.20	
fracture	1.40	NS	2.25	<0.01
osteotomy	1.15	NS	3.41	<0.05
Proximal marrow				
cast	1.01		1.02	
fracture	0.62	<0.02	0.98	NS
osteotomy	1.20	<0.01	0.88	NS
Distal marrow				
cast	1.27		1.21	
fracture	0.45	<0.001	0.97	NS
osteotomy	1.02	<0.02	1.15	NS
Skeletal muscle				
cast	1.27		1.17	
fracture	1.85	NS	1.80	NS
osteotomy	1.36	NS	2.14	NS

[a] Cast immobilization alone (Triffitt et al. 1992)
[b] Fracture by blunt trauma
[c] Surgical osteotomy (Triffitt et al. 1993)
[d] Not significant at 5 percent level

flow is not clear, but the recovery of flow by 2 weeks suggests that a temporary effect, such as marrow edema, was responsible. This may have arisen from the division of the marrow by tearing rather than by the lesser trauma of an osteotomy.

The limitation of the increase in distal cortical flow seen at 2 weeks on comparison with osteotomy thus appears to have arisen from damage to the paraosseous tissues. As the rise in muscle flow was not reduced after blunt trauma, it is probable that periosteal stripping from the cortex was responsible.

A standard experimental fracture, an osteotomy with minimal soft tissue dissection, might be considered to be a model of a very low energy injury. The results seen after the higher energy fractures in this study suggest that such injuries depress the expected blood flow response, and this lends support to a vascular theory of delayed union. Further investigation will be required to confirm that this depression is sufficient to have a material effect on long-bone fracture healing.

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