VASCULAR ENDOTHELIAL GROWTH FACTOR ENHANCES BONE HEALING IN AN EXPERIMENTAL NONUNION MODEL

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INTRODUCTION
Reestablishment of vascularity is an early event in fracture healing, and upregulation of angiogenesis may therefore promote bone formation in avascular sites or where angiogenesis is limiting osteogenesis. Fractures at avascular sites often proceed to nonunion. Deposition of an angiogenic growth factor at the fracture site could induce angiogenesis and subsequently bone formation thus preventing nonunion formation. The most potent angiogenic growth factor is recombinant human vascular endothelial growth factor (rhVEGF). It stimulated angiogenesis in experiment settings, and clinically, intramyocardial injection increased myocardial blood flow and relieved ischaemic symptoms in coronary patients (2).

We hypothesized that local administration of rhVEGF could augment bone formation by increasing blood flow at the osteotomy site in an experimental hypovascular nonunion model.

METHODS
Rabbits, divided into 3 groups of 8, underwent a standard nonunion operation, that included mid-tibial osteotomy, removal of periostium and endostium proximal and distal to the osteotomy site, and plate fixation by a 5-hole 5/8 plate. Then 100µg rhVEGF delivered in a 1% hyaluronan gel carrier, carrier alone, or autograft, harvested from the iliac crest, was deposited into the osteotomy gap before wound closure.

Evaluation of the osteotomy area after 7 weeks comprised blood flow estimation by radioactive microsphere deposition, destructive torsional testing, micro-CT scans, radiography and callus weight and size.

The comparisons rhVEGF vs. Control group, and rhVEGF vs. Autograft group was done by Non-parametric Mann-Whitney U test for 2 unpaired samples, and p < 0.05 indicated a significant difference.

The experiments were approved by the danish committee on animal experimentation (approval #2002/561-501).

RESULTS
rhVEGF treated and autografted osteotomies united, whereas carrier treated osteotomies failed to unite (Figure 1). Torsional failure moment and cross sectional area of the callus at the osteotomy site were significantly larger in rhVEGF group than in the carrier group, but not different from the autografted osteotomies.

Then 100µg rhVEGF delivered in a 1% hyaluronan gel carrier, carrier alone, or autograft, harvested from the iliac crest, was deposited into the osteotomy gap before wound closure.

Median failure moment was 1500 Nmm (1100-1850), 400Nmm (0-1021), and 1300Nmm (1200-1400) in the rhBMP-2, control, and autograft group respectively, and callus cross sectional area were 54mm² (43-61), 39mm² (8-48), and 55mm² (52-60) respectively (25% and 75% quartiles in brackets).

Blood flow at the rhVEGF treated osteotomy sites were not different from the carrier and autografted osteotomy sites. Median bone blood flow in the united osteotomies were 16 ml/min/100g.

Micro-CT derived histomorphometric parameters showed a lower bone volume fraction in rhVEGF treated osteotomies than in the autografted osteotomies.

DISCUSSION
The osteotomy gap was deprived its normal osteoprogenitor cell supply through stripping of periostium and endostium, and in this environment, local stimulation by rhVEGF delivered in a hyaluronan gel carrier stimulated bone formation.

As VEGF has no osteoinductive capacity itself, rhVEGF-induced vessels must have repopulated the osteotomy gap and provided to the gap the growth factors and bone forming cells necessary for competent endochondral bone formation.

The possibility to initiate and amplify the bone forming cascade through a purely angiogenic stimulus could be used clinically to prevent nonunion formation in fractured hypovascular bone, such as fractured irradiated or infected bone or fractures associated with severe soft tissue damage.

Our results demonstrated that rhVEGF is a potential bone graft substitute. The model has previously been shown histologically to mimic atrophic nonunions (1). Bone blood flow in united osteotomies, 7 weeks after the operation, were not different from the united osteotomies indicating that established nonunions were highly perfused. Ischaemia and avascularity may therefore play an early role in the pathogenesis of nonunion, but not in maintenance of the nonunion state.

REFERENCES