Enhanced tendon healing with GDF 5 and 6

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Between ruptured tendon ends, undifferentiated mesenchymal cells invade the hematoma and differentiate to form a tendon regenerate. This differentiation is partly directed by mechanical stimuli, which are difficult to apply and control clinically. For example, closed treatment of Achilles tendon ruptures is associated with a risk of rerupture of the regenerate. Improved tendon healing by exogenous growth factors has not previously been reported. Three proteins in the Bone Morphogenetic Protein (BMP) family—namely Growth and Differentiation Factors (GDFs) 5, 6 and 7—have recently been shown to induce a tendon- or ligament-like tissue after intramuscular implantation in rats, indicating a new way to improve tendon healing. We transected the Achilles tendon in 66 rats and denervated the calf muscle. Denervation served to reduce the mechanical stimulation to the tendon callus by eliminating muscle contractions. GDF 5 or 6 were implanted on collagen sponges in the tendon defects in two doses and compared to collagen sponges alone. The rats were killed after 2 weeks and the tensile strength of the tendon regenerate was found to be increased by both proteins in a seemingly dose-dependent manner.

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Bone Morphogenetic Proteins constitute a family of multifunctional proteins which can induce bone formation from mesenchymal stem cells. They are morphogens, having many roles during the development of the skeleton and other organs. With rising concentration thresholds, they can initiate chemotaxis, proliferation, differentiation and cartilage or bone formation (Reddi, 1997). Three Growth and Differentiation Factors in this family, GDF 5, 6 and 7 (Chang et al. 1994, Storm et al. 1994), have recently been shown to induce a tendon- or ligament-like tissue rather than cartilage and bone, after intramuscular implantation in rats (Wolfman et al. 1997). Although others report bone induction by GDFs in similar models, it is clear that GDFs have less osteogenic potential than, e.g., OP-1/BMP7 in doses which are equipotent for effects on cartilage matrix synthesis (Erlacher et al. 1998). GDFs 5 and 6 are also called Cartilage-Derived Morphogenetic Proteins (CDMP) 1 and 2.

In non-surgically treated Achilles tendon ruptures in humans, a functional tendon regenerate forms within 6–8 weeks, but the time until full recovery is many months, and there is a risk of rerupture (Nistor 1981). One cause of low tensile strength of a tendon regenerate is insufficient mechanical stimulation during healing (Murrell et al. 1994). This has led to attempts to use controlled loads during regeneration by physiotherapy (McComis et al. 1997). Another option would be to inject into the early tendon defect hematoma a factor which could direct tissue development towards a tendon-like tissue, even in the absence of mechanical stimulation. Just as Bone Morphogenetic Proteins are expected to have a successful future in fracture treatment, a tendon inducer might be valuable in the treatment of tendon ruptures and other pathological conditions in tensile structures.

We applied GDF 5 or 6 to transected Achilles tendons in rats and measured the strength of the regenerate. To simulate the immobilized human situation in the rats, mechanical stimulation by muscle contraction was reduced by transection of the tibial nerve.

Animals and methods

67 female Sprague-Dawley rats (about 200 g; Møllegård, Copenhagen) were allocated to treatment groups, according to the Table. They were anesthetized with chloral hydrate intraperitoneally (4 mg/kg body weight). A 3 mm transverse skin incision was made beside the right Achilles tendon. The surrounding fascia was split longitudinally and the Achilles tendon complex was dissected free. Since the plantaris tendon is well developed in rats, it was removed to prevent it from acting as an internal splint. The Achilles tendon was cut about 5 mm proximal to the calcaneal insertion and the skin was sutured. A 15 mm long posterolateral incision was then made just above the

Allocation of rats to treatment groups

Treatment	Mechanical	Histology	Discarded	Total
Control	13	0	6	19
GDF5 1 µg	7	5	2	14
GDF6 1 µg	9	5	0	14
GDF5 10 µg	9	0	1	10
GDF6 10 µg	8	0	2	10
Total	46	10	11	67

knee. The tibial nerve was identified and cut 1–2 mm proximal to the branches to the calf muscles. Thus, the peroneal nerve was spared.

1 × 2.5 × 2.5 mm pieces of collagen (Helistat™) were prepared aseptically from larger pieces. Human recombinant GDF5 and GDF6 were expressed in E. coli (Creative Biomolecules, Hopkinton, MA, USA) and given to us after lyophilization from an acetonitril and tetrafluoroacetic acid solution. The lyophilized proteins were dissolved in water and 0, 1 or 10 µg of GDF5 or GDF6 in 10 µL solution was soaked on the collagen, which was then lyophilized. When the Achilles tendons had been cut, a 3–5 mm defect formed, in which the collagen sponges were placed.

The rats were killed after 14 days. 5 specimens from each 1µg group were taken for routine histology, using longitudinal paraffin sections and HE stain. The lower limbs of the other animals were frozen in liquid nitrogen. They were then wrapped in gauze soaked with physiological saline and stored in a –20 °C freezer. After about 1 week, the specimens were thawed in physiological saline at room temperature. The calcaneus and Achilles tendon were dissected. For mechanical testing, the muscle was gently scraped off, so that the proximal tendon formed a thin blade, which was sandwiched between pieces of a paper towel and fixed in a metal clamp. The calcaneus was fixed in a specially devised clamp, so that the angle between the calcaneus and Achilles tendon corresponded to 30° dorsiflexion of the foot. The clamps were fixed in a material-testing machine built for this study. A transducer was built from a brass ring and strain gauges in a 4-bridge, and connected to a computer to measure the force applied momentarily. The system was calibrated with weights. The tendon was pulled at a speed of 1.0 mm/s, without preconditioning.

Results

11 specimens were discarded before the tensile test, because of macroscopic cracks in the tissue that had formed by freezing. 6 of these were controls (Table). Failure loads increased from median 17 N to 23 and 25 N with 10 µg of GDF 5 and 6, respectively (Kruskal-Wallis for all 5 groups: $p = 0.003$; Figure 1). Both GDFs at 10 µg and GDF6 at 1µg differed from controls (Mann-Whitney for each < 0.02).

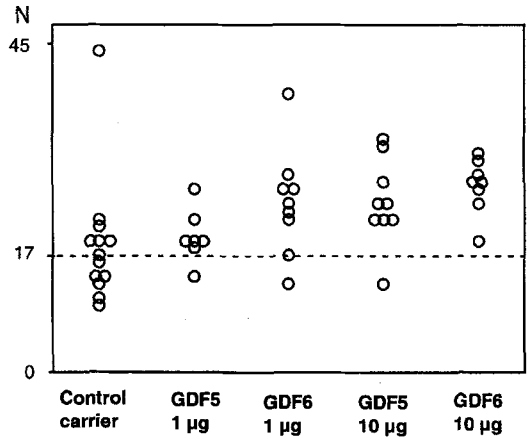


Figure 1. Tension at failure (N) of rat Achilles tendon regenerates 14 days after transsection, tibial denervation and implantation of collagen sponges (carrier) with GDFs 5 or 6. Median value of controls (17 N) marked by dotted line.

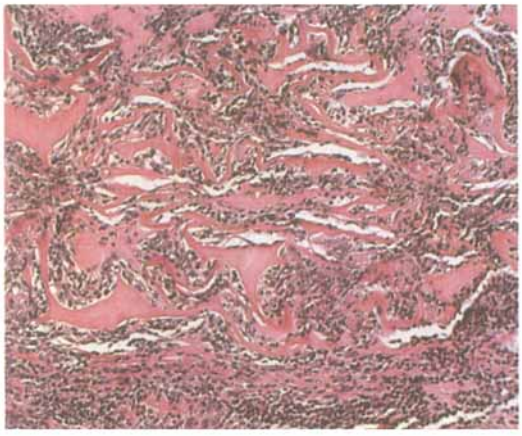
The GDF-treated specimens differed from controls macroscopically in color and had a firmer consistency on palpation (unblinded observations).

In all 10 GDF-treated specimens, histology revealed cell-rich areas in the center of the defect, interspersed with remnants of the implanted collagenous bundles. This was not seen in 10 identically treated control specimens in previous experiments used for comparisons (Figure 2). In one specimen treated with GDF6, this GDF-specific area contained small amounts of hyaline cartilage, but in all GDF specimens it was surrounded by a tendon regenerate of normal appearance.

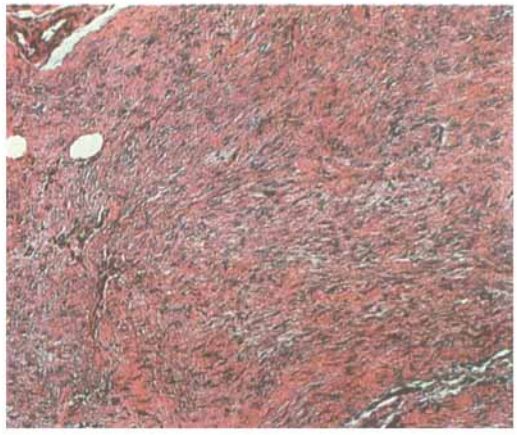
Discussion

We developed this rat model some years ago in futile attempts to stimulate tendon healing with less specific mitogens, such as FGF2 and TGFß1. Growth and Differentiation Factors 5 and 6 appear to have a more morphogenic function and act in a new way, by specifically directing differentiation (Wolfman et al. 1997). With these factors, a more traction-resistant tendon regenerate was induced. It remains to be seen whether this effect can be increased by still higher doses and also whether an injection regime is possible. Bone morphogenetic proteins are now used as

Figure 2.



A. Tendon regenerate treated with 1 µg of GDF6. Remnants of collagen implant with cell-rich infiltrate. This is surrounded by dense new fibrous tissue as seen in B.



B. Tendon regenerate treated with control solution. Collagen implant has been resorbed and new fibrous tissue is forming.

implants in clinical series on treatment of fractures and lumbar fusions, without reported systemic side-effects (Geesink and Bulstra 1997). We have also managed to reproducively induce bone formation by a single local injection of OP-1/BMP7 (Forslund and Aspenberg 1998). It therefore seems possible that GDF injections will prove useful.

OP-1 in our tendon model induces bone formation, which leads to a decreased tensile strength, and doses below the bone inductive threshhold do not increase tendon strength (unpublished data). Therefore the effect of the GDFs appears to be specific, and not a general effect of the BMP family of proteins. However, GDFs have been shown to induce cartilage and bone in a model (Erlacher et al. 1998) very similar to that in which Wolfman et al. found only a tendon-like tissue (Wolfman et al. 1997). This may indicate that the response to the GDFs is highly dependent on local factors, such as mechanical signals, to direct the differentiation pathway. Indeed, this is true also of BMP2 and OP-1, which not only can stimulate bone fracture healing, but also reduce it, depending on factors such as the severity of trauma and mechanical stimulation (Jeppsson and Aspenberg 1996, Bostrom et al. 1997).

We found an increase in strength of more than a third with 10 µg injections. If this can be repeated in larger animals, injections of GDFs may change Achilles tendon ruptures from a condition that is usually operated on, to one that is treated with injections in the outpatient clinic. The principle of applying a morphogen that directs the wound-healing response towards forming a tensile-resistant tissue might also become a useful adjunct to treatment of other conditions with failure of tensile structures.

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