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Nutrition of cruciate ligament reconstruction by diffusion

Collagen synthesis studied in rabbits

The reconstructed anterior cruciate ligament was studied in the rabbit using the medial third of the patellar tendon. Tritiated proline, 100 μCi/kg body weight, was injected intra-articularly to insure detection of the metabolic conversion product ^{3}H-hydroxyproline in the avascular graft. During the immediate postoperative period, nutrients were found to derive from the synovial fluid through a process of diffusion, demonstrating that synovial nutrition occurs prior to revascularization of the graft.

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Several animal studies of patellar tendon substitution of the anterior cruciate ligament have documented the process of graft incorporation (Alm et al. 1974, Arnoczky et al. 1982, Cabaud et al. 1980). A synovial source of these nutrients has been suggested on the basis of hydrogen washout investigations (Ginsburg et al. 1980, Whiteside & Sweeney 1980). The objective of our study was to ascertain whether nutrients normally present within the synovial fluid are utilized by the cells of the patellar tendon used to reconstruct the anterior cruciate ligament in the rabbit.

Materials and methods

The anterior cruciate ligament (ACL) was reconstructed in the left knees of nine New Zealand white male rabbits using the medial third of the patellar tendon. The rabbits were anesthetized with ketamine 100 mg/kg and xylazine 20 mg/kg intramuscularly. Antibiotic prophylaxis of 100,000 units of penicillin was given i.m. A medial parapatellar incision was used to expose the quadriceps and patellar tendon, extending from 2 cm proximal to the patella to the tibial tubercle. A 4.5 × 0.5 cm strip of tendon was sharply dissected from its proximal origin and was left attached to the tibia. A Bunnel-type suture with 4-0 prolene was placed in the free end of the graft. The knee was placed in extension to dislocate the patella and then hyperflexed to allow incision of the fat pad and exposure of the ACL. The ACL was then removed from the joint with sharp dissection and 3-mm drill holes were placed through the medial tibia and lateral femoral condyle, which entered the joint at the site of the excised ligament. The autograft was pulled through the drill holes by straightening the needle and placing it within the hollow tip of an 18-gauge hypodermic needle, which was passed through the joint. The needle was then remodeled to its original shape and used to sew the autograft, held taut at 40° of flexion, to the periosteum of the lateral femoral condyle. The joint capsule and quadriceps defects were closed with a running simple stitch of 4-0 prolene. The skin was closed with 3-0 ethilon running simple stitch. The animals were then returned to caged, non-immobilized activity.

After a recovery period of 1 week, the animals were anesthetized using ketamine and rompum i.m. Tritiated proline (spec. act. 22 Ci/mmole, 1 mCi/ml) from a single batch was injected intraarticularly into the left knee at a dose of 100 μCi/kg body weight by manual pressure in 30 sec. A high-precision Hamilton syringe was used, inserting the needle into the suprapatellar pouch of the synovial cavity and directing the tip down the patellar groove. Following the injection the joint was moved through its full range of motion ten times. An average volume of 0.35 ml was felt to provide sufficient isotope to 1) insure detection of the metabolic conversion product, ^{3}H-hydroxyproline; and 2) achieve rapid distribution of ^{3}H-proline throughout the joint. Also renografin arthrograms were performed, using the injection method described, to demonstrate the distribution of the 0.35 ml injectate.

An unoperated control group of six rabbits had the left knee of each animal injected with tritiated proline using identical doses and injection techniques described for the experimental group.

Forty-eight hours after injection, the intra-articular portion of the graft was harvested and analyzed biochemically. Isolation of hydroxyproline was achieved by cation-exchange liquid chromatography, and quantitation of ^{3}H-hydroxyproline was achieved by determining its radioactivity using liquid scintillation spectrometry (Akeson et al. 1977). The amount of total hydroxyproline (representative of collagen content) was determined by the method of Woessner (1961). Relative amounts of ^{3}H-hydroxyproline, or the amount of collagen synthesized during the 48-hr period, were expressed as counts per minute of ^{3}H-hydroxyproline per microgram of total hydroxyproline (specific radioactivity).

Results

The injection technique was evaluated arthrographically to ascertain the distribution of the isotope within the joint. After injecting and ranging the joint 10 times, the dye was seen at the edges of the joint, with the excess remaining in the suprapatellar pouch. The radiopaque blush faded with time and had disappeared within 15 min. Therefore, the injectate volume was sufficient to fill the synovial cavity, and ^{3}H-proline uptake could be correlated with synovial fluid access.

At the time of killing, all knees were free of infection. The knees were mildly swollen, but had a full passive range of motion. There were no patellar dislocations and no synovial fistulas. The grafts were all intact and glistening white in gross appearance, similar to their appearance intraoperatively. A mild synovitis was characteristically present, with blood-tinged synovial fluid.

Analysis of the tissues showed that the collagen content in the unoperated control patellar tendons and grafted patellar tendons was 86.2 ± 1.0 and 85.7 ± 1.2 per cent, respectively. Tritiated hydroxyproline was found to be present in both control and graft tissues, although its concentration in the grafts was approximately tenfold higher than in the control. This finding indicates a significantly elevated rate of collagen synthesis in the grafted tendons during the 48-hour postoperative time period ($P < 0.05$, two-tailed t test).

Discussion

Proline was chosen because it is a normal constituent of synovial fluid (Bauer et al. 1940), and it is also an essential nutrient in collagen synthesis. Furthermore, incorporation of ^{3}H-proline into the collagen matrix of the patellar tendon graft may be detected by analyzing the graft for ^{3}H-hydroxyproline resulting from hydroxylation of proline during collagen synthesis (White et al. 1973).

The presence of radioactivity within the avascular graft can only be explained by diffusion of the tritiated proline from the synovial cavity into the graft. The intra-articular transfer of the patellar tendon resulted in a greater uptake of tritiated proline, which in part may be due to greater accessibility to synovial nutrients. The conversion of proline to hydroxyproline in the process of collagen synthesis requires the activity of the enzyme prolyl hydroxylase, an intracellular process occurring in fibroblasts, dependent on O_2, Fe^{2+}, α-ketoglutarate, and ascorbic acid (White et al. 1973). Hydroxyproline synthesis indicates that the graft is metabolically active prior to being revascularized, and derives proline, and presumably other nutrients, from the synovial fluid through a process of diffusion.

Further support for diffusion is suggested by the observation of Arnoczky and associates (1982) that cells proliferated on the periphery of avascular patellar tendon grafts, with central necrosis. Matthews (1976), Eiken and associates (1975), Lundborg & Rank (1978), and Lundborg and associates (1980) made similar observations in avascular free grafts of flexor tendon, noting peripheral cellular proliferation with degeneration in the deepest areas. Whiteside & Sweeney (1980) noted that in an anterior cruciate ligament left floating free within the suprapatellar pouch of the knee, the surface layer cells thickened. It seems plausible that nutrient diffusion from synovial fluid may support cell metabolism in the periphery of the graft, but that central necrosis occurs in regions beyond a critical depth. A need for nu-

trients therefore exists within the depths of the graft, and a teleologic answer to this may be vascular ingrowth within the graft (Noyes 1980).

Passive diffusion is a process dependent on several factors, including concentration of molecules, temperature, size and motion of the molecules, and properties of the membrane through which diffusion occurs (Guyton 1971). In man, compared with the rabbit, patellar tendon grafts are quite large and, although it may be inferred that similar diffusion of synovial nutrients occurs in man, the diffusion process will be limited by size, and relatively less tendon graft will be exposed to synovial nutrients. Factors that increase passive diffusion may be expected to increase nutrient delivery. Motion may not only enhance synovial fluid production, but may in effect stir the synovial nutrients, thereby aiding diffusion. This may be the basis behind the benefits of continuous passive motion.

In our model, synovial nutrient delivery cannot be quantitated. Because long-term survival of patellar tendon grafts appears to depend on revascularization (Alm et al. 1974, Arnoczky et al. 1982, Clancy et al. 1981), it is doubtful that synovial nutrition alone is able to maintain a functional graft.

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