

RANDOMIZED PROSPECTIVE DOUBLE-BLIND PLACEBO-CONTROLLED STUDY OF DEXTROSE PROLOTHERAPY FOR KNEE OSTEOARTHRITIS WITH OR WITHOUT ACL LAXITY

Kenneth D. Reeves, MD, and Khatab Hassanein, PhD

Kenneth D. Reeves is the section chief for Physical Medicine and Rehabilitation at Bethany Medical Center, Kansas City, Kan. **Khatab Hassanein** is professor and chairman for the Department of Biometry at the University of Kansas Medical Center, Kansas City, Kan.

Context • Use of prolotherapy (injection of growth factors or growth factor stimulators).

Objective • Determine the effects of dextrose prolotherapy on knee osteoarthritis with or without anterior cruciate ligament (ACL) laxity.

Design • Prospective randomized double-blind placebo-controlled trial.

Setting • Outpatient physical medicine clinic.

Patients or other participants • Six months or more of pain along with either grade 2 or more joint narrowing or grade 2 or more osteophytic change in any knee compartment. A total of 38 knees were completely void of cartilage radiographically in at least 1 compartment.

Intervention • Three bimonthly injections of 9 cc of either 10% dextrose and .075% lidocaine in bacteriostatic water (active solution) versus an identical control solution absent 10% dextrose. The dextrose-treated joints then received 3 further bimonthly injections of 10% dextrose in open-label fashion.

Main Outcome Measures • Visual analogue scale for pain and swelling, frequency of leg buckling, goniometrically measured flexion, radiographic measures of joint narrowing and osteophytosis, and KT1000-measured anterior displacement difference (ADD).

Results • All knees: Hotelling multivariate analysis of paired observations between 0 and 6 months for pain, swelling, buckling episodes, and knee flexion range revealed significantly more benefit from the dextrose injection ($P = .015$). By 12 months (6 injections) the dextrose-treated knees improved in pain (44% decrease), swelling complaints (63% decrease), knee buckling frequency (85% decrease), and in flexion range (14 degree increase). Analysis of blinded radiographic readings of 0- and 12-month films revealed stability of all radiographic variables except for 2 variables which improved with statistical significance. (Lateral patellofemoral cartilage thickness [$P = .019$] and distal

NOTICE: THIS MATERIAL MAY BE
PROTECTED BY COPYRIGHT LAW
(TITLE 17, U.S. CODE)

femur width in mm [$P = .021$]. Knees with ACL laxity: 6-month (3 injection) data revealed no significant improvement. However, Hotelling multivariate analysis of paired values at 0 and 12 months for pain, swelling, joint flexion, and joint laxity in the dextrose-treated knees, revealed a statistically significant improvement ($P = .021$). Individual paired t tests indicated that blinded measurement of goniometric knee flexion range improved by 12.8 degrees ($P = .005$), and ADD improved by 57% ($P = .025$). Eight out of 13 dextrose-treated knees with ACL laxity were no longer lax at the conclusion of 1 year.

Conclusion • Prolotherapy injection with 10% dextrose resulted in clinically and statistically significant improvements in knee osteoarthritis. Preliminary blinded radiographic readings (1-year films, with 3-year total follow-up period planned) demonstrated improvement in several measures of osteoarthritic severity. ACL laxity, when present in these osteoarthritic patients, improved. (*Altern Ther Health Med.* 2000;6(1):68-80)

INTRODUCTION

Prolotherapy (injection of growth factors or growth factor stimulators) raises growth factor levels or increases growth factor effectiveness to promote tissue repair or growth. The most common solutions used for prolotherapy create a brief inflammatory response. Temporary cellular stress causes a release of cytokines and increased growth factor activity with migration of macrophages (white blood cells), and then multiplication of repair cells specific to the tissue. Unlike repair after an injury, disruption of architecture of tissue from injury does not occur, and new cells and matrix can be deposited in an organized fashion, with maturation of new tissue for 6 to 8 weeks.¹ Two double-blind studies have been performed on prolotherapy in low back pain using inflammatory solutions.^{2,3} These studies both showed significant benefit from proliferant injection, but because the solutions were inflammatory there was some potential for impairment of double-blind protocol. The purpose of this investigation was to evaluate effectiveness of prolotherapy without using any inflammatory mechanism so that neither patient, research coordinator

Reprint requests: InnoVision Communications, 101 Columbia, Aliso Viejo, CA 92656; phone, (800) 899-1712 or (949) 448-7370 (ext 515); fax, (949) 362-2049; e-mail, iv@reprint.com.

nor primary investigator would have any way to determine patient group. Our specific plan was to study the effect of a non-inflammatory (10%) concentration of dextrose (D-glucose in water) on knee osteoarthritis patients via objective measures of knee cartilage, knee osteophytic status, and knee goniometric range, as well as by subjective measures of knee pain, knee swelling, and knee buckling.

Some patients in the study had anterior cruciate ligament (ACL) laxity, which is known to initiate and worsen knee osteoarthritis. A second purpose for this study was to observe the effect of proliferant injection on laxity of the ACL, as measured by an objective and reproducible measure (an electroarthrometer).

Elevation of extracellular glucose to as little as .5% (normal extracellular and cellular glucose is .1%) has been shown to raise levels of multiple polypeptide growth factors in a variety of human cells.⁴⁻⁶ Exposure of several human cells to a hypertonic environment will also promptly result in a rise in DNA levels for growth factors within seconds to minutes.^{9,10} Therefore, hypertonic dextrose solution has 2 mechanisms by which to increase levels of growth factors, potentially improving the status of critical cells in the joint such as chondrocytes (cartilage producing cells), osteocytes (bone producing cells), and fibroblasts (tendon/ligament/other soft tissue producing cells).

METHODS

Ads were placed for patients with knee arthritis to receive injection of a solution to reduce pain in knee osteoarthritis. Criteria for knee osteoarthritis included 6 months or more of pain in the knee, accompanied by either grade 2 or more joint narrowing or grade 2 or more osteophytic change. Grade 2 joint narrowing can be described as the presence of less than or equal to 3 mm of cartilage (found in only 8% without symptomatic knee osteoarthritis [OA]).¹¹ A grade 2 osteophyte can be described as a short, fat and obvious bone spur or a moderately long (10 mm or more), thin bone spur (found in only 14% without symptomatic knee OA).¹¹ A standard radiographic atlas was used to determine joint narrowing and osteophytic grades, which was designed for that purpose.¹²

The ability to verify ACL laxity by any arthrometer requires testing of both knees for an anterior displacement difference (ADD) side to side. Using this method, the KT1000 (Medmetric Corporation, San Diego, Calif) has been shown to be equal to or more reliable than other arthrometers.¹³⁻¹⁶ Based on extensive review of previous studies of the KT1000 an ADD of 2 is estimated to be 85% sensitive and 85% specific for ACL laxity.^{15,17,18} Since this study was not funded to allow for magnetic resonance imaging (MRI) studies to rule out complete ACL tear, the number of patients with complete ACL tear could not be determined. Note that the objectivity of this electroarthrometer is found in its use of standard positioning of the knee within the device, audible indications when certain pressures are applied to the knee through the device, a precise readout easily visible for recording, and a routine to perform each reading 3 times to average all 3 readings.

Once the patients were found to meet radiologic and symp-

tomological criteria for knee osteoarthritis, they were assigned serially to group 1 or 2 using a random number table by 1 of 2 data base coordinators always in the office. This group assignment was kept in a database blinded to the chief investigator and research coordinator.

The research coordinator obtained an estimate of arthritis medications taken and then demonstrated the use of a 100-mm visual analogue scale (VAS) and gave 3 examples of its use. The patients then self-scored their pain levels of knee pain at rest, knee pain walking on level surfaces, knee pain with stair use, and subjective swelling, and estimated the number of knee buckling episodes over the previous 2 months. Following this, the research coordinator obtained goniometric readings of joint flexion by the method described in a standard text.²⁰

Patients who were taking any medication or oral supplement for osteoarthritis other than calcium, multivitamins, NSAIDS, acetaminophen, or occasional narcotic, were asked to discontinue them. The most common oral supplement discontinued was glucosamine/chondroitin sulfate.

Blood was obtained for sedimentation rate, rheumatoid factor, uric acid, and antinuclear antibody. Significant laboratory abnormalities led to referral to primary physician or rheumatologist for determination of the presence or absence of inflammatory arthritis. No patients required exclusion due to the laboratory battery after the initial phone screening.

Dextrose prolotherapy solutions for maximum safety have typically included bacteriostatic water, a small concentration of lidocaine, and dextrose. Because of the desire to maximize safety and comfort in this study and simulate typical prolotherapy solutions, the control was the usual bacteriostatic water with a very small amount of lidocaine, and the active solution was identical except for the inclusion of 10% dextrose.

At 0, 2, and 4 months solution was drawn up blinded to both chief investigator and research coordinator. Using a 27-gauge needle via an inferomedial approach, tibiofemoral injection was conducted with 9 cc of either 611.4 mOsm (10% dextrose and .075% lidocaine in bacteriostatic water) or 105.4 mOsm (.075% lidocaine in bacteriostatic water) solution. Bacteriostatic water consisted of .9% benzyl alcohol. The small dose of lidocaine was included for postinjection comfort. The solutions were identical in color and viscosity. Dextrose at 10% concentration is very slightly sticky if allowed to dry on the skin but Hibiclenz was used for glove and skin prep which masked any potential of noting any slight stickiness of solution.

Treatment continued beyond 6 months in the dextrose group with additional injections at 6, 8, and 10 months. Subjective variables, goniometric flexion, 2-view radiographs and KT-1000 ADD measurements were repeated at 1 year. Skier's (standing) views of the knee were used to determine tibiofemoral compartment status. Angle of knee flexion on skier's views, angle of radiograph beam to the knee, camera to film distance, power and duration of radiograph beam, and radiograph technician were identical at 0 and 12 months. Magnification on standing films was prevented by ensuring contact of patella with film plate. Skyline views of the patella

were used to determine patellofemoral compartment status, with similar measurements, including camera to knee and knee to film distance, to ensure an identical radiograph method.

Radiographs were read in double-blind fashion in the following way. The study coordinator obscured patients' names and labeled the film with a random patient number. The film date was obscured and a random number table was used to assign a number to the 0- and 12-month films. The 0- and 12-month films were then separated in different packets so that reading 1 film would not influence reading of the next. Osteophytic grade was measured in 6 compartments using a standard atlas with approximately 90% intra-reader agreement.¹² The compartments included medial femoral, medial tibial, lateral femoral, lateral tibial, medial patellofemoral, and lateral patellofemoral. Cartilage thickness was determined in 4 compartments in millimeters: medial tibiofemoral, lateral tibiofemoral, medial patellofemoral, and lateral patellofemoral. General hypertrophic change was evaluated as a width measurement in millimeters: distal femur width proximal to the intercondylar notch, distal femur width distal to the intercondylar notch, and proximal tibial width. Width measurements were made parallel with the film bottom edge through the area of largest width, including any osteophytes present. The x-rays were read by the chief investigator. A database coordinator loaded results onto the database.

Human subject research approval and monitoring was by the Institutional Review Committee of Bethany Medical Center in Kansas City, Kans. Procedures followed were in accordance with ethical standards outlined in the Helsinki Declaration Revision of 1983. The statistical analysis software was SPSS (Statistical Program for Social Science) version 7.5.3.

RESULTS

Blinding method problems were not identified. No treatment complications were noted. Seventy-seven patients had 1 or more knees that met study criteria for symptomatic osteoarthritis (OA). Nine patients dropped out over 12 months of followup, 4 due to lack of efficacy (3 in control group and 1 in active group), and 5 for unrelated medical reasons. This left 111 knees in 68 patients with OA.

At study onset 31 patients met arthrometric criteria for ACL laxity. Two dropped out over 12 months due to lack of efficacy and 4 for unrelated medical issues, leaving 25 for analysis.

Independent sample *t* tests were conducted to compare the active and control groups of OA knees. No significant differences were noted between groups for age, weight, pain levels, range of motion, buckling episodes, or radiographic findings. The same result was noted when *t* tests were conducted to compare active and control groups of knees with ACL laxity. The average knee OA patient in this study was 63 years of age and weighed 195 lb. Males comprised 58% of the study population.

Complications and Safety Issues

Discomfort after injection did not appear to vary between groups, typically lasting a few minutes to several days.

Despite use of an allergy-size needle (27 gauge) and a single-insertion technique, some patients had pain with distension of the joint capsule even with this minimal volume (9 cc). The 9 cc volume for injection may be a bit excessive in that some patients were inhibited in flexion for several days. One person had a flare postinjection that appeared substantial, requiring interarticular steroid and then referral to an orthopedic surgeon. When blinding was broken she was found to have received control solution.

No allergic reactions or infections were noted.

Six-Month (Double-Blind Phase) Data Comparing Active and Control Solution for all Osteoarthritic Knees

Figure 1 presents a bar graph depicting improvements in pain (average of improvement in pain at rest, pain with walking, and pain with stair use) and swelling for the active and control groups at 6 months (after 3 injections of 9 cc of solution). Both active and hypotonic control solution administration resulted in considerable gains in VAS scores for pain.

Figure 2 shows improvement in knee flexion for both groups at 6 months. Both active and hypotonic control injections resulted in an improvement in goniometric knee flexion measures.

Hotelling multivariate analysis of paired observations between 0 and 6 months for active and control solution including all nonradiographic variables (pain at rest, pain with walking, pain with stair use, swelling, buckling episodes, and flexion range) demonstrated a statistically superior effect of active solution ($P = .015$). The results of individual paired *t* tests from 0 to 6

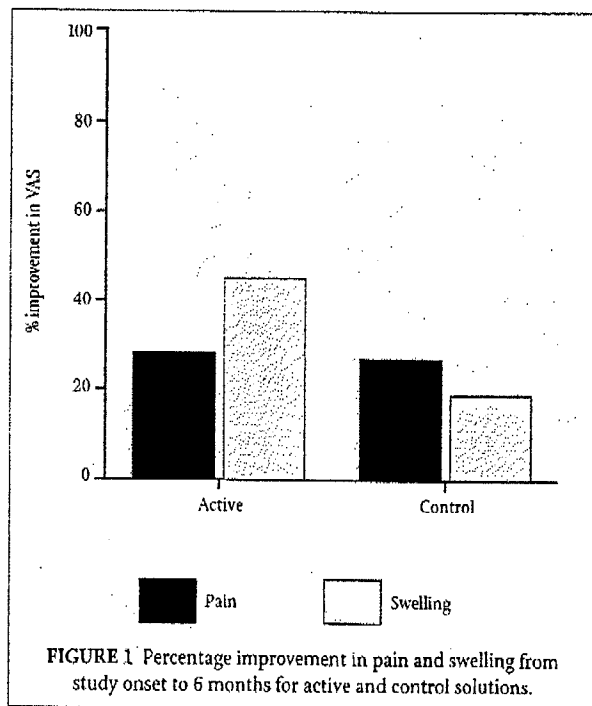


FIGURE 1 Percentage improvement in pain and swelling from study onset to 6 months for active and control solutions.

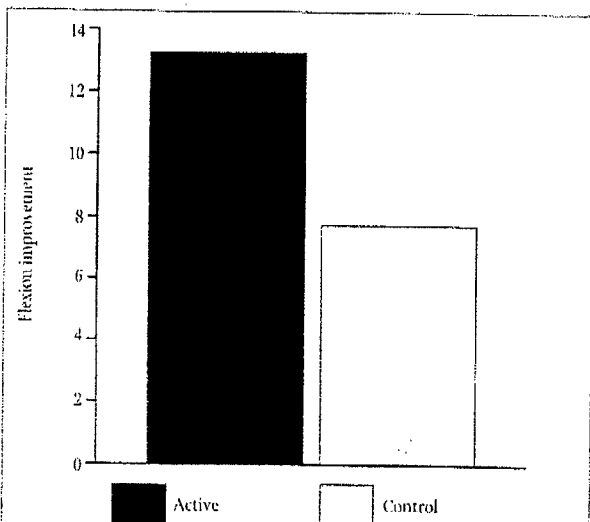


FIGURE 2 Degree improvement in knee flexion range of motion after 3 bimonthly injections of active or control solution.

months for each of the variables are shown in Table 1. Although the active solution was superior statistically, highly significant improvement from 0 to 6 months was seen in pain with walking, pain with stair use, and flexion range of motion in both active and control groups.

The NSAID follow-up question was limited in its ability to determine a degree of change in level of intake, and no significant change between groups was noted. However, neither group had an increase in NSAID intake, which could explain improvement in pain levels or other variables.

One-Year Data (Nonradiographic) for Active Solution for Osteoarthritic Knees

Figure 3 shows percentage improvements in pain and swelling complaints and knee buckling in the dextrose group between 0 and 12 months (with 3 further bimonthly open label injections of dextrose). Pain improved by 40%, swelling by 63%, buckling episodes by 85%, and flexion by 14 degrees as compared with study entry.

Radiographic Data at 1 Year for Active Solution for Osteoarthritic Knees (Table 2)

Thirteen radiographic readings for each knee are shown in Table 2. These variables included medial femoral osteophyte grade (MFOG), medial tibial osteophyte grade (MTOG), lateral femoral osteophyte grade (LFOG), lateral tibial osteophyte grade (LTOG), medial patellofemoral osteophyte grade (MPOG), lateral patellofemoral osteophyte grade (LPOG), medial tibiofemoral cartilage thickness (MTFT), lateral tibiofemoral cartilage thickness (LTFT), medial patellofemoral cartilage thickness (MPFT), lateral patellofemoral cartilage thickness (LPFT), distal femur

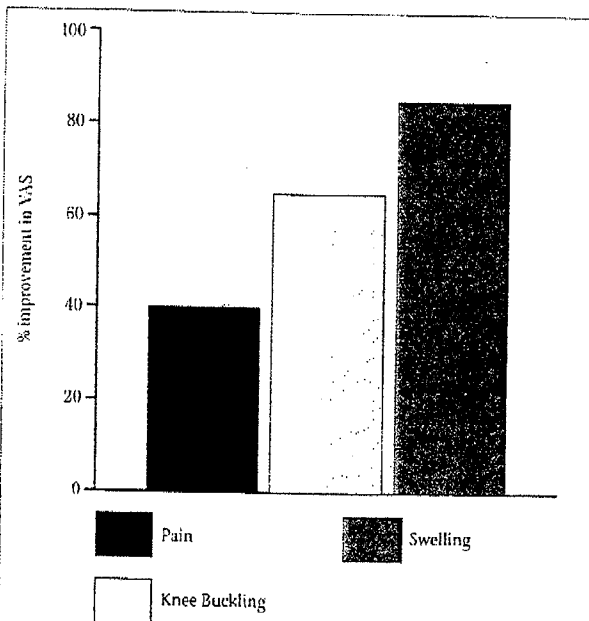


FIGURE 3 Percentage improvement in pain, subjective swelling and number of knee buckling episodes after 6 injections of active solution (at 1-year follow-up).

width proximal to the intercondylar notch (DFWP), distal femur width distal to the intercondylar notch (DFWD), and proximal tibial width (PTW).

Hotelling multivariate analysis of paired observations between 0 and 12 months for the dextrose-treated knees including all 13 radiographic variables revealed a statistically significant change ($P=.028$). Individual paired t tests showed the means for radiographic variables were all stable except for an improvement (increase) in lateral patellofemoral cartilage thickness ($P=.019$) and an improvement (decrease) in distal femur width including osteophytes ($P=.021$).

Data for Knees with ACL Laxity

The 6-month data showed no statistically significant differences between active and control solutions, nor significant changes in ACL laxity measurement. However, the dextrose-treated knees were given 3 additional injections of dextrose and data were collected at 1-year follow-up. Hotelling multivariate analysis of paired observations of the dextrose-treated knees comparing 0 and 12 months for VAS rest pain, VAS walking pain, VAS stair use pain, VAS swelling complaint, flexion range of motion, and KT1000 side-to-side difference showed statistically significant improvement over time ($P=.021$). The results of individual paired t tests from 0 to 12 months are shown in Table 3. Blinded goniometric range measurement improved by 12.8 degrees with a P value of .005 and KT1000 ADD improved by 57% with a P value of .025. Figure 4 is a bar graph showing the

TABLE 1 Means, standard deviations (SD), and individual paired *t* tests for change in nonradiographic variables from 0 to 6 months in all osteoarthritic knees for active and control solution

	Group	Mean (SD) 0 months	Mean (SD) 6 months	Mean diff 0-6 months	Standard error of mean diff	95% CI for the mean difference	Significance between means at 0 and 6 months
Pain at rest	Active	2.15 (2.24)	1.61 (1.71)	-.54	.24	-1.02 to -.06	.029
	Control	2.73 (2.02)	1.69 (1.73)	-1.04	.25	-1.54 to -.54	.00005
Pain with walking	Active	3.94 (2.82)	2.56 (1.97)	-1.39	.31	-2.01 to -.77	.00002
	Control	3.83 (2.20)	2.85 (2.20)	-.98	.32	-1.62 to -.34	.003
Pain with stair use	Active	5.33 (2.80)	3.96 (2.68)	-1.37	.32	-2.01 to -.73	.00004
	Control	5.83 (2.60)	4.60 (2.91)	-1.23	.32	-1.87 to -.59	.0002
Swelling	Active	2.44 (2.53)	1.35 (1.87)	-1.09	.25	-1.59 to -.59	.00003
	Control	3.12 (2.99)	2.52 (2.80)	-.60	.26	-1.12 to -.08	.022
Buckling episodes per 2 months	Active	7.78 (34.14)	2.54 (11.44)	-5.24	2.23	-9.70 to -.78	.020
	Control	1.00 (2.60)	.21 (.64)	-.79	2.27	-5.33 to +3.75	.729
Flexion range	Active	112.35 (19.54)	125.59 (8.63)	-13.24	2.15	+8.94 to +17.54	.00000001
	Control	117.75 (11.32)	125.44 (7.48)	-7.69	2.19	+3.31 to +12.07	.001

distribution frequency of laxity values (ADD) for dextrose-treated patients at time 0 and 12 months. Note that 8 of the 13 improved to the point that ADD was less than 2, such that they would no longer be considered lax, and this in a group of patients with one or more complete ACL ruptures.

DISCUSSION

Balance of Growth and Disrepair Factors in Bony Cortex, Cartilage, and Synovial Fluid

The balance of disrepair and repair in both bone and cartilage merit examination since degenerative changes in bone occur simultaneously with those in cartilage.^{21,22}

Chief repair factors found in osteoarthritic subchondral bone or cartilage include insulin-like growth factor (IGF), transforming growth factor beta (TGF- β), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and platelet derived growth factor (PDGF).^{21,22} Chief disrepair factors (factors that block growth factor effects or break down tissue or building blocks for tissue) for the bony surface or cartilage include interleukin-1 (IL-1) and tumor necrosis factor (TNF), which lead to a rise as much as 110-fold in metalloproteinases such as collagenase (which breaks down cartilage) in fibrillated cartilage and a rise as much as 24-fold in binding proteins (proteins that bind growth factors to keep them from functioning) in synovial fluid.²³⁻²⁷

TABLE 2 Means, standard deviations (SD), and individual paired *t* tests for change in radiographic variables from 0 to 12 months in osteoarthritic knees treated with active solution

Variable	Mean (SD) 0 months	Mean (SD) 6 months	Mean diff 0 - 12 months	Standard error of mean diff	95% CI for the mean difference	Significance between means at 0 and 6 months	Direction of change
MFOG	1.55 (1.07)	1.49 (1.02)	-.06	.11	-.28 to +.16	NS	Stable
MTOG	1.56 (.92)	1.53 (1.05)	-.03	.10	-.23 to +.17	NS	Stable
LFOG	1.65 (.91)	1.76 (.84)	+.11	.13	-.15 to +.37	NS	Stable
LTOG	1.22 (.95)	1.33 (1.00)	+.11	.13	-.15 to +.37	NS	Stable
MPOG	1.24 (.82)	1.25 (.82)	-.01	.12	-.25 to +.23	NS	Stable
LPOG	1.42 (.66)	1.40 (.66)	-.02	.08	-.18 to +.14	NS	Stable
MTFT	2.09 (2.18)	1.94 (2.14)	-.15	.13	-.41 to +.11	NS	Stable
LTFT	5.54 (2.06)	5.58 (2.32)	+.04	.17	-.30 to +.38	NS	Stable
MPFT	4.51 (1.63)	4.59 (1.41)	+.08	.19	-.30 to +.46	NS	Stable
LPFT	4.20 (1.54)	4.59 (1.34)	+.39	.16	+.07 to +.71	.019	Improved
DFWP	93.58 (7.23)	92.96 (7.07)	-.62	.26	-1.14 to -.10	.021	Improved
DFWD	90.18 (8.36)	90.60 (8.14)	+.42	.34	-.26 to +1.10	NS	Stable
PTW	89.18 (7.96)	88.53 (8.08)	-.65	.39	-1.43 to +.13	NS	Stable

Proliferation of Human Chondrocytes by Growth Factors in Culture and Chondrogenesis of Animal Cartilage by Injection of Growth Factors

Bujia²⁸ and Dunham²⁹ demonstrated that culturing human chondrocytes (nasal septum chondrocytes) in fluid containing TGF- β ,²⁸ IGF-I,²⁹ or bFGF^{28,29} resulted in proliferation. Injection of animal knees with a single injection of TGF- β ,³⁰ bone metabolic protein-2 (BMP-2),³⁰ bFGF,³¹ or hepatocyte growth factor (HGF)³² has led to chondrogenesis,³⁰ enlargement of articular cartilage,³¹ and repair of full thickness joint cartilage defects.³²

Implantation of gel or a collagen sponge saturated with growth factor or placement of a surgically placed small pump that delivers growth factors have led to repair of full thickness cartilage lesions in animal models, also.^{31,35} However, demonstration of 3 weeks of proteoglycan synthesis after a single injection of TGF- β ³⁰ and healing of full-thickness cartilage lesions with a single injection of growth factor³² indicates that continuous exposure to growth factor may not be required for a prolonged growth factor effect. In established OA high levels of binding proteins or metalloproteinases may block the effect of a single growth factor

TABLE 3 Means, standard deviations (SD), and individual paired *t* tests for change in pain, swelling, flexion, and laxity variables from 0 to 12 months for active solution in knees with ACL laxity

	Mean (SD) 0 months	Mean (SD) 12 months	Mean diff 0-12 months	Standard error of mean diff	95% CI for the mean difference	Significance between means at 0 and 12 months
Pain at rest	2.31 (2.56)	1.38 (2.06)	-.93	.49	-1.91 to +.06	.082
Pain with walking	3.77 (2.77)	2.31 (2.72)	-1.46	.46	-2.38 to -.92	.008
Pain with stair use	5.54 (3.31)	4.15 (3.29)	-1.39	.47	-2.33 to -.45	.013
Swelling	2.77 (2.71)	1.54 (2.40)	-1.23	.66	-2.55 to +.09	.088
Flexion range	112.69 (16.93)	125.46 (6.89)	+12.77	3.77	+5.23 to +20.31	.005
KT1000 side to side diff	3.08 (1.32)	1.23 (2.24)	-1.85	.72	-3.29 to -.41	.025

injection, and there may be a need in humans to combine growth factors with agents that neutralize disrepair factors for optimum effectiveness in established osteoarthritis.²⁵

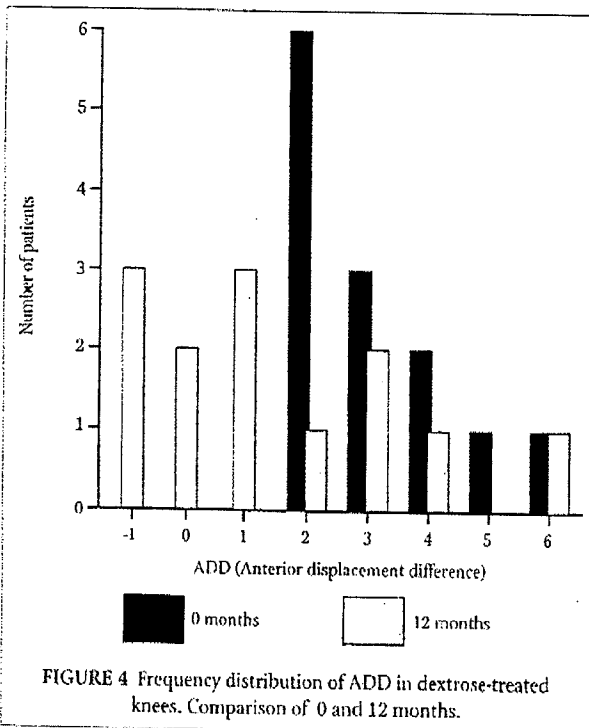


FIGURE 4 Frequency distribution of ADD in dextrose-treated knees. Comparison of 0 and 12 months.

Proliferation of Human Fibroblasts by Growth Factors in Culture

Human ACL ligament growth factors have not been fully elucidated, but Marui et al²⁶ demonstrated in cell suspension that collagen production by the human ACL ligament cell is increased by transforming growth factor beta (TGF- β) and epidermal growth factor beta (EGF- β), with EGF- β the most potent.

Effect on Human Cells of Exposure to Elevated Glucose

Elevation of extracellular glucose to as little as .5% has been shown to raise levels of IGF-1 in human mesangial (glomerular) cells,⁷ IGF-2 in human mesangial cells,⁷ TGF- β 1 in human mononuclear cells⁸ and human mesangial cells,¹⁷ PDGF-B (platelet derived growth factor beta) in human mesangial cells⁴ and human capillary endothelial cells,¹⁷ bFGF in human gingival fibroblasts,⁶ and connective tissue growth factor (CTGF) in human mesangial cells.³ In addition, glucose in blood mononuclear cells has been found to suppress potential disrepair factors (interleukins such as IL-2, IL-6, and IL-10).⁸ Cellular response to elevated extracellular glucose is swift. DNA levels for growth factor production rise within minutes to hours of cellular exposure to elevated glucose concentrations.¹⁸ As many as 15 different genes are induced with exposure to elevated glucose concentration.³

Effect on Human Cells of Exposure to Osmolar Changes

Exposure of a cell to an osmolarity change as little as 50 mOsm has also been found to activate enzymes (phosphate donors, also termed kinases) in the cell similar to the growth factors mentioned above.^{9,10,39-42} The mechanism appears to be via a

change in cell size, leading to kinase production via natural cellular responses to stress.^{41,44} Although the kinases produced by osmolar change are not the same as with glucose elevation, proliferation response to a change in osmolarity has been demonstrated and at least 1 kinase produced is clearly a growth factor related to proliferation (PDGF).¹⁰

Potential Therapeutic Benefit of Bacteriostatic Water Solution or Anesthetic

The osmolarity of the bacteriostatic water solution used for the control injection was 105 compared to 611 for the active group. Information about the potential efficacy of hypotonic solution came out in the literature after our study began. This raised the question of whether the hypotonic control solution in this study was more than a placebo treatment. Review of placebo responses in recent double-blind studies of knee osteoarthritis revealed a range of pain reduction from 9% to 30%.⁴⁵⁻⁴⁹ Review of studies on knee OA in which knee flexion measurements were obtained before and after treatment yielded few studies. A search over the last 30 years indicated a range of improvement in knee flexion in placebo groups from a -4.6 degree loss to a 1 degree gain.⁵⁰⁻⁵³ The control group in this study improved by 28% in pain and 8 degrees in flexion range, suggesting more than a placebo effect. Since ligaments in different locations in animals respond to different growth factors there may be dissimilar findings for different joints.⁵⁴⁻⁵⁶ Thus it is of interest that a concurrent finger OA study did show similar benefit with dextrose solution but the control solution did not show an appreciable benefit.³⁷

It is possible that there was some therapeutic effect from inclusion of anesthetic in the solution as well, and if so this may explain some benefit in both groups. However, the concentration of lidocaine at .075% was quite low and was identical in both treatment solutions.

Magnitude of Clinical Benefit from Dextrose Solution Use Compared to Active Groups in Other Recent Studies

Pain improvement in the active treatment group by 40% through 1 year after 6 injections of 9 cc of simple dextrose solution approximated that of the active treatment group in recent studies on avocado soybean unsaponifiables,^{48,58} chondroitin sulfate,⁵⁹ glucosamine,⁴⁷ and NSAIDs.^{46,53,60,61} Range of motion improvement in flexion in the dextrose-treated knees (14 degrees) exceeded the range of flexion improvement (-2.6 to +12.5) in active treatment groups found in double-blind knee arthritis studies over the past 30 years.⁵⁰⁻⁵³ No past studies could be found that quantified subjective swelling complaints or knee buckling frequency to compare with the 63% and 85% reductions demonstrated in the current study. Only 2 other studies indicated potential stabilization of radiograph findings similar to the current study.^{62,63}

Previous Prolotherapy Injection Trials on Knee Ligament Laxity

Double-blind studies of injection prolotherapy with non-inflammatory solutions for knee osteoarthritis or knee ligament

laxity have not been previously reported. However, stimulation of the inflammatory cascade produces growth factors, and temporary inflammation induction by sodium morrhuate has been shown in a double-blind study in rabbits to thicken and strengthen knee collateral ligaments.⁶⁴ The only human study on knee-ligament strengthening by inflammatory induction (using a 1.25% phenol 12.5% dextrose and 12.5% glycerine solution) had few patients and was unblinded.⁶⁵ However, despite the low patient numbers, highly significant improvement of laxity measurements by a Genucom knee arthrometer was noted.

Potential Applications and Future Study Implications

This study is 1 of 2 concurrent double-blind studies (along with a concomitant finger arthritis study)³⁷ to demonstrate that 10% dextrose alone is capable of a beneficial effect upon introduction into OA joints and that a treatment frequency of every 2 months is effective. Potential applications include patients too large or too young for total knee replacement, any patient in a third world country without replacement availability, patients who are symptomatic despite prescribed exercises or physical therapy or NSAIDs, or patients who are intolerant of NSAIDs.

This is the first study to demonstrate in double-blind fashion that simple 10% dextrose will correct ACL ligament laxity in an objectively-measurable fashion. Potential applications may include patients with laxity without rupture, post surgical repair to prevent the typical post-surgical gradual loosening, and large total joint patients with dislocation tendency.⁶⁶ The ability to intervene in a simple way for ACL laxity to limit the known complications of secondary arthritis should be of much interest. The broadness of application of dextrose injection in ligament/tendon treatment will depend on the cost of alternative treatments such as growth-factor-impregnated implants, direct stem cell injection, or injection of ACL ligament cells transfected with viruses whose genome has been altered to produce growth factors or to block growth factor inhibitors.^{67,68} The safety and low cost of dextrose injection may make it suitable for study in prophylactic use for knee injection in athletes prone to ACL injuries or in those with injuries but intact ligament.

These study results with 10% dextrose use are intriguing in that clinical experience indicates that dextrose 25% is superior to 10% dextrose in the treatment of knee OA and ACL laxity. This author is currently investigating the ability of patients to tell the difference between 10% and 25% dextrose upon injection into the knee in preparation for direct study of 25% dextrose, to be certain that double-blind protocols would not be affected by the brief inflammatory effect of 25% dextrose. Future study protocols using dextrose for prolotherapy should consider different volumes of dextrose injection, as some have suggested that smaller volumes are equally effective and may allow 25% dextrose to be used without patient awareness. Other applications of injection prolotherapy and areas of past and current study are covered in 2 recent publications.^{71,72}

If growth factor production results in more inexpensive and safe solutions for injection, this may be an alternative to stimulating growth factors by either brief inflammation or by dextrose

or by osmotic effects, and yet a likely outcome is that oral supplements, growth factor stimulant injection (prolotherapy), and direct growth factor provision by injection or other method will be complementary.

Frequency of treatment necessary for dextrose injection needs further evaluation, with current studies not designed to answer all questions about this. Clinical experience with 25% dextrose suggests that 2 to 3 bimonthly treatments are necessary prior to treatment taper.

For future studies on the ACL ligament, MRI availability to rule out complete ACL rupture and arthroscopy to confirm changes in cartilaginous surfaces would be ideal.

Now that the safety of dextrose in bacteriostatic water has been demonstrated in this study and a concomitant finger osteoarthritis study, future studies with dextrose should perhaps have dextrose in sterile water or saline versus an isotonic saline placebo.

Long-term radiograph follow-up data from the current study patients will be helpful to note net effect on cartilage and osteophytic change over a prolonged period, and patients are being followed for long-term radiographic findings.

CONCLUSIONS

Dextrose injection is clinically and statistically superior to bacteriostatic water in treatment of OA of the knee, with substantial improvements in joint pain, subjective joint swelling, flexion range of motion, and tendency for knee buckling. Anterior cruciate ligament tightening by objective measures was demonstrated with use of interarticular dextrose. Preliminary (1-year) radiographic findings show positive effects but 30- to 36-month followup radiography is planned for a clearer idea of the effect of proliferant injection on radiographic findings of OA. The inclusion of 38 knees in this study that were completely void of cartilage in at least 1 compartment, the long history of pain (8 years) in these knee OA patients, and their average size (195 lbs) strengthen the significance of the clinical outcomes demonstrated.

This study is remarkable in part because it represents an effective intervention with injection of as little as 9 cc of simple dextrose injection on 3 separate occasions. This study result, coupled with findings of a double-blind study on small joint (finger) OA, indicates that dextrose injection may have broad effectiveness in the treatment of joint and soft tissue.⁵⁷ Future studies using isotonic saline as placebo and using a higher concentration of dextrose solution will be important, although blinding may be more difficult for such studies. In the meantime prolotherapy with dextrose should be considered as one of the treatments for OA of knee and ACL laxity.

References

1. Banks AR. A rationale for prolotherapy. *J Orthop Med (UK)*. 1991;13(3):54-59.
2. Klein RG, Bjorn CE, DeLong R, Mooney V. A randomized double-blind trial of dextrose-glycerine-phenol injections for chronic low back pain. *J Spinal Disord*. 1993;6:23-33.
3. Ongley MJ, Klein RG, Dorman TA, Eck BC. A new approach to the treatment of chronic low back pain. *Lancet*. 1987;2:143-146.

4. DiPaolo S, Gesualdo L, Ranieri E, Grandaliano G, Schena FP. High glucose concentration induces the overexpression of transforming growth factor-beta through the activation of a platelet-derived growth factor loop in human mesangial cells. *Am J Pathol*. 1996;149(6):2095-2106.
5. Murphy M, Godson C, Cannon S, et al. Suppression subtractive hybridization identifies high glucose levels as a stimulus for expression of connective tissue growth factor and other genes in human mesangial cells. *J Biol Chem*. 1999;274(9):5830-5839.
6. Ohgi S, Johnson PW. Glucose modulates growth of gingival fibroblasts and periodontal ligament cells: correlation with expression of basic fibroblast growth factor. *J Periodontol Res*. 1996;31(8):579-588.
7. Pugliese G, Pricci F, Lucuratolo N, et al. Increased activity of the insulin-like growth factor system in mesangial cells cultured in high glucose conditions. Relation to glucose-enhanced extracellular matrix production. *Diabetologia*. 1996;39(7):775-784.
8. Reinhold D, Ansorge S, Schleicher ED. Elevated glucose levels stimulate transforming growth factor-beta 1 (TGF-beta 1), suppress interleukin IL-2, IL-6 and IL-10 production and DNA synthesis in peripheral blood mononuclear cells. *Horm Metab Res*. 1996;29(5):267-270.
9. Krump E, Nikitas K, Grinstein S. Induction of tyrosine phosphorylation and Na+/H-exchanger activation during shrinkage of human neutrophils. *J Biol Chem*. 1997;272(28):17303-11.
10. Okuda Y, Adrogue HJ, Nakajima T, et al. Increased production of PDGF by angiotensin and high glucose in human vascular endothelium. *Life Sci*. 1996;59(17):1465-1461.
11. Felson DT, McAlindon TE, Anderson JJ, et al. Defining radiographic osteoarthritis for the whole knee. *Osteoarthritis Cartilage*. 1997;5:241-250.
12. Altman RD, Hochberg M, Murphy Jr WA, Wolfe F, Lequesne M. Atlas of individual radiographic features in osteoarthritis. *Osteoarthritis Cartilage*. 1995;3(suppl A):3-70.
13. Bach B, Warren R, Flynn W, et al. Arthrometric evaluation of knees that have a torn anterior cruciate ligament. *J Bone Joint Surg Am*. 1990;72-A:1299-1306.
14. Daniel D, Malcom L, Lusse C, et al. Instrumented measurement of anterior laxity of the knee. *J Bone Joint Surg Am*. 1985;67A:720-726.
15. Liu S, Osti L, Henry M, Bocchi L. The diagnosis of acute complete tears of the anterior cruciate ligament: comparison of MRI, arthrometry and clinical examination. *J Bone Joint Surg Br*. 1995;77-B(4):586-588.
16. Steiner M, Brown C, Zarins B, et al. Measurement of anterior-posterior displacement of the knee: a comparison of the results with instrumented devices and with clinical examination. *J Bone Joint Surg Am*. 1990;72A:1307-1315.
17. Daniel D, Stone M, Sachs R, Malcom L. Instrumented measurement of knee laxity in patients with acute anterior cruciate ligament disruption. *Am J Sports Med*. 1985;13(6):401-407.
18. Highgenboten C, Jackson A, Jansson K, Meske N. KT-1000 arthrometer: conscious and unconscious test results using 15, 20 and 30 pounds of force. *Am J Sports Med*. 1992;20(4):450-454.
19. Wrohle R, Van Ginkel L, Grood E, Noyes F, Shaffer B. Repeatability of the KT 1000 Arthrometer in a normal population. *Am J Sports Med*. 1990;18(4):396-399.
20. Erickson RP, McPhee MC. Clinical Evaluation. In: DeLisa JA, Gans BM, Currie DM, et al, eds. *Rehabilitation Medicine Principles and Practice*. 2nd ed. Philadelphia, Pa: J.B. Lippincott; 1993:62-69.
21. Brandt KD. Osteoarthritis. In: Fauci AS, Braunwald E, Isselbacher KJ, et al, eds. *Harrison's Principles of Internal Medicine*. 14th ed. New York, NY: McGraw Hill; 1998:1936-1937.
22. Hough Jr AJ. Pathology of osteoarthritis. In: McCarty DJ, Koopman WJ, eds. *A Textbook of Rheumatology*. 12th ed. Philadelphia, Pa: Lea and Febiger; 1993:1709-1721.
23. Castor CW, Smith EM, Bignall MC, Hossler PA. Connective tissue activation. XXXVII. Effects of cytokine combinations, implications for an integrated cytokine network. *J Rheumatol (Canada)*. 1997;24(11):2080-9.
24. Dequeker J, Mokassa L, Aertssens J, Boonen S. Bone density and local growth factors in generalized osteoarthritis. *Microm Res Tech*. 1997;37(4):358-371.
25. Hilal G, Marrel-Pelletier J, Pelletier JP, Ranger P, Lajeunesse D. Osteoblast-like cells from human subchondral osteoarthritic bone demonstrate an altered phenotype in vitro: possible role in subchondral bone sclerosis. *Arthritis Rheum*. 1998;41(5):891-899.
26. Olney RC, Tsuchiya K, Wilson DM, et al. Chondrocytes from osteoarthritic cartilage have increased expression of insulin-like growth factor I (IGF-I) and IGF binding protein-3 (IGFBP-3) and -5, but not IGF-II or IGFBP-4. *J Clin Endocrinol Metab*. 1996;81(3):1096-1103.
27. Tsuchiya K, Maloney WJ, Yu T, et al. Osteoarthritis: differential expression of matrix metalloproteinase-9 mRNA in nonfibrillated and fibrillated cartilage. *J Orthop Res*. 1997;15(1):94-100.
28. Bujla J, Pitzke P, Kastanbauer E, Wilmes E, Hammer C. Effect of growth factors on matrix synthesis by human nasal chondrocytes cultured in monolayer and in agar. *Eur Arch Otorhinolaryngol*. 1996;253(6):336-340.
29. Dunham BP, Koch RJ. Basic fibroblast growth factor and insulinlike growth factor I support the growth of human septal chondrocytes in a serum-free environment. *Arch Otolaryngol Head Neck Surg*. 1998;124(12):1325-1330.
30. Van Beuningen HM, Glansbeek HL, van der Kraan PM, van den Berg WB. Differential effects of local application of BMP-2 or TGF-beta 1 on both articular cartilage composition and osteophyte formation. *Osteoarthritis Cartilage*. 1998;6(5):306-317.
31. Shida J, Jingushi S, Izumi T, Iwaki A, Sugioka Y. Basic fibroblast growth factor stimulates articular cartilage enlargement in young rats in vivo. *J Orthop Res*. 1996;14(2):265-272.
32. Wakitani S, Imoto K, Kimura T. Hepatocyte growth factor facilitates cartilage repair. Full thickness articular cartilage defect studied in rabbit knees. *Acta Orthop Scand*. 1997;68(5):474-480.

33. Grgic M, Jelic M, Basic V, Baic N, Pecina M, Vukicevic S. Regeneration of articular cartilage defects in rabbits by osteogenic protein-1 (bone morphogenetic protein-7). *Acta Med Croatica*. 1997;51(1):23-27.
34. Otsuka Y, Mizuta H, Takagi K, et al. Requirement of fibroblast growth factor signaling for regeneration of epiphyseal morphology in rabbit full-thickness defects of articular cartilage. *Dev Growth Differ*. 1997;39(2):143-156.
35. Sellers RS, Peluso D, Morris EA. The effect of recombinant human bone morphogenetic protein-2 (rhBMP-2) on the healing of full-thickness defects of articular cartilage. *J Bone Joint Surg Am*. 1997;79(10):1452-1463.
36. Marui T, Niyibizi C, Georgescu H, et al. Effect of growth factors on matrix synthesis by ligament fibroblasts. *J Orthop Res*. 1997;15(1):18-23.
37. Inaba T, Ishizashi S, Gotoda T. Enhanced expression of platelet-derived growth factor-beta receptor by high glucose. Involvement of platelet-derived growth factor in diabetic angiopathy. *Diabetes*. 1998;45(4):507-512.
38. Oh JH, Ha H, Yu MR, Lee HB. Sequential effects of high glucose on mesangial cell transforming growth factor-beta 1 and fibronectin synthesis. *Kidney Int*. 1998;54(6):1872-1878.
39. Beal T, Sivirivardana G, Ao J, Butterfield LM, Heasley LE. Multiple mitogen activated protein kinases are regulated by hyperosmolality in mouse IMCD cells. *Am J Physiol*. 1997;272(3 pt 2):305-311.
40. Caruccio L, Bae S, Liu AY, Chen KY. The heat-shock transcription factor HSF1 is rapidly activated by either hyper- or hypo-osmotic stress in mammalian cells. *Biochem J*. 1997;323(2):341-347.
41. Ruis H, Schuller C. Stress signaling in yeast. *Bioessays*. 1995;17(11):959-965.
42. Szasz K, Buday L, Kapus A. Shrinkage-induced protein tyrosine phosphorylation in Chinese hamster ovary cells. *J Biol Chem*. 1997;272(26):16670-16678.
43. Sadoshima J, Izumo S. Cell swelling rapidly activates src tyrosine kinase, a potential transducer of mechanical stress in cardiac myocytes. *Circulation*. 1996;94(suppl 1):409.
44. Sadoshima J, Qui Z, Morgan J, Izumo S. Tyrosine kinase activation is an immediate and essential step in hypotonic cell swelling-induced ERK activation and c-fos gene expression in cardiac myocytes. *EMBO J*. 1996;15(20):5535-5546.
45. Bocanegra TS, Weaver AL, Tindall EA, et al. Diclofenac/misoprostol compared with diclofenac in the treatment of osteoarthritis of the knee or hip: a randomized, placebo controlled trial. Arthrotec osteoarthritis study group. *J Rheumatol (Canada)*. 1998;25(8):1602-1611.
46. Gordon A, Merenstein JH, D'Amico F, Hudgens D. The effects of therapeutic touch on patients with osteoarthritis of the knee. *J Fam Pract*. 1998;47(4):271-277.
47. Leffler CT, Philipp AF, Leffler SC, Masure JC, Kim PD. Glucosamine, chondroitin, and manganese ascorbate for degenerative joint disease of the knee or low back: a randomized, double-blind, placebo-controlled pilot study. *Mil Med*. 1999;164(2):85-91.
48. Maheu E, Mazieres B, Valat JP, et al. Symptomatic efficacy of avocado/soybean unsaponifiables in the treatment of osteoarthritis of the knee and hip: a prospective, randomized, double-blind, placebo-controlled, multicenter clinical trial with a six-month treatment period and a two-month followup demonstrating a persistent effect. *Arthritis Rheum*. 1998;41(1):81-91.
49. Stuckel NA, Day RO, Keller B, Brooks PM. Copper-salicylate gel for pain relief in osteoarthritis: a randomized controlled trial. *Med J Aust*. 1997;4:167(3):134-136.
50. Gramajo RJ, Cutrona EJ, Fernandez DE, et al. A single-blind, placebo-controlled study of glycosaminoglycan-peptide complex (Rumalon) in patients with osteoarthritis of the hip or knee. *Curr Med Res Opin*. 1989;11(6):366-373.
51. Katona G. A clinical trial of glycosaminoglycan-peptide complex (Rumalon) in patients with osteoarthritis of the knee. *Curr Med Res Opin*. 1987;10(9):625-633.
52. Vajradul Y. Double-Blind Clinical Evaluation of intra-articular glucosamine in outpatients with gonarthrosis. *Clin Ther*. 1981;3(5):336-342.
53. Perez Busquier M, Calero E, Rodriguez M, et al. Comparison of aceclofenac with piroxicam in the treatment of osteoarthritis. *Clin Rheumatol (Belgium)*. 1997;18(2):154-159.
54. Lee JD, Hwang O, Kim SW, Han S. Primary cultured chondrocytes of different origins respond differently to bFGF and TGF-beta. *Life Sci*. 1997;61(3):293-299.
55. Kang HJ, Kang ES. Ideal concentration of growth factors in rabbit's flexor tendon culture. *Yonsei Med J (Seoul)*. 1999;40(1):26-29.
56. Scherping SC Jr, Schmidt CC, Georgescu H, Kwok CK, Evans CH, Woo SL. Effect of growth factors on the proliferation of ligament fibroblasts from skeletally mature rabbits. *Connect Tissue Res (England)*. 1997;36(1):1-8.
57. Reeves KD, Hassancin K. Randomized prospective double blind placebo controlled study of dextrose prolotherapy for osteoarthritic thumb and finger (DIP, PIP and trapezometacarpal) joints: evidence of clinical efficacy. *J Altern Complement Med*. 2000. In press.
58. Blotman F, Maheu E, Wutwik A, Caspard H, Lopez A. Efficacy and safety of avocado/soybean unsaponifiables in the treatment of symptomatic osteoarthritis of the knee and hip: a prospective, multicenter, three-month, randomized, double-blind, placebo-controlled trial. *Rev Rhum Ed Fr*. 1997;64(12):825-834.
59. Bucsi L, Poor G. Efficacy and tolerability of oral chondroitin sulfate as a symptomatic slow-acting drug for osteoarthritis (SYSADOA) in the treatment of knee osteoarthritis. *Osteoarthritis Cartilage*. 1998;May;6 suppl A:31-36.
60. Lund B, Distel M, Bluhmki E. A double-blind, randomized, placebo-controlled study of efficacy and tolerance of meloxicam treatment in patients with osteoarthritis of the knee. *Scand J Rheumatol*. 1998;27(1):32-37.
61. Wu JJ, Shih LY, Hsu HC, Chen TH. The double-blind test of sodium hyaluronate (ARTZ) on osteoarthritis knee. *Chung Hua I Hsueh Tsa Chih (Taipei)*. 1997;58(2):99-106.
62. Conrozier T. Anti-arthritis treatments: efficacy and tolerance of chondroitin sulfates (CS 486). *Presse Med*. 1998;27(36):1862-1865.
63. Uebelhart D, Thonar EJ, Delmas PD, Chantraine A, Vignon E. Effects of oral chondroitin sulfate on the progression of knee osteoarthritis: a pilot study. *Osteoarthritis Cartilage*. May 1998;6 suppl A:39-46.
64. Liu Y, Tipton C, Matthus R, Bedford T, Maynard J, Walmer H. An in-situ study of the influence of a sequestering solution in rabbit medial collateral ligaments and its junction strength. *Conn Tiss Res*. 1983;11:95-102.
65. Ongley M, Dorman T, Eck B, Lundgren D, Klein R. Ligament instability of knees: a new approach to treatment. *Manual Medicine*. 1988;3:152-154.
66. Reeves K, Harris A. Recurrent dislocation of total knee prostheses in a large patient: case report of dextrose proliferant use. *Arch Phys Med Rehabil*. 1997;78:1039. Abstract.
67. Kobayashi D, Kurosaka M, Yoshiya S, Mizuno K. Effect of basic fibroblast growth factor on the healing of defects in the canine anterior cruciate ligament. *Knee Surg Sports Traumatol Arthrosc*. 1997;5(3):189-194.
68. Dye S. The future of anterior cruciate ligament restoration. *Clin Orthop*. 1996;325:130-139.
69. Gerich T, Kang R, Fu F, Robbins P, Evans C. Gene transfer to the rabbit patellar tendon: potential for genetic enhancement of tendon and ligament healing. *Gene Ther*. 1996;3(12):1089-1093.
70. Pelletier J, Caron J, Evans C, et al. In vivo suppression of early experimental osteoarthritis by interleukin-1 receptor antagonist using gene therapy. *Arthritis Rheum*. 1997;40(6):1012-1019.
71. Reeves K. Prolotherapy: present and future applications in soft tissue pain and disability. *Phys Med Rehab Clin North Am*. 1995;6(4):917-926.
72. Reeves K. Prolotherapy: Basic science, clinical studies and technique. In: Leonard JA, ed. *Pain Procedures in Clinical Practice*. 2nd ed, Philadelphia, Pa: Hanley and Belfus; 2000:172-190.

"I teach at Saybrook because of the rigor of the course work, the flexibility of the program and the ingenuity and creativity of the mature, student body."



Jeanne Achterberg, Ph.D

SAYBROOK

Graduate School & Research Center

www.saybrook.edu

800-825-4480