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1. Chart Review Study of Subjects Administered Amnionic Membrane for Treatment of Joint Pain

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Abstract
Context: Treatment of joint pain with injection of amnionic membrane has not been adequately studied. ***Objectives:*** Determine if patients who received cryopreserved particulate amnionic membrane (CPAM) injected into painful back and knee joints experience less back or knee pain, improve physical ability, and use less opiates and NSAIDs over a 12 week time period. ***Methods:*** Charts were reviewed for 20 consenting patients receiving CPAM, clinically available from tissue banks, injected into joints to relieve pain consistent with the clinical practice at a single center. Ten subjects had back pain, and 10 subjects had knee pain. ***Results:*** VAS pain scores improved from 7.5 to 1.1 over 12 weeks (p<0.001). WOMAC daily activity function score improved from 46 to 11over 12 weeks (p<0.001). Opiate usage decreased from 55% to 15% over 12 weeks (p<0.001). NSAID usage decreased from 80% to 10% over 12 weeks (p<0.001). Location of injection was not a significant covariate factor for any outcome. ***Conclusion:*** Thus, amnionic membrane injection into painful back and knee joints improves pain and physical function, and decreases opiate and NSAID usage for at least 12 weeks.

Key Words : *Amnion, amnionic membrane, joint pain, knee pain, back pain*

Introduction

Joint pain remains a significant debilitating problem affecting over 27 million Americans. OA is associated with ongoing inflammation, oxidative stress and activation of matrix metalloproteases (MMP) that culminate in cartilage degradation (1). Back pain accounts for 149 million work days lost, $40 billion in annual costs, 3 million ER visits costing $9.5 billion and estimated impact of $200 billion per year (2). Of knee and hip OA patients, 25% cannot perform major daily activities and 40% report fair to poor health, ranking high in disability adjusted life years with total knee replacements costing $28.5 billion (3, 4). Existing treatments for joint pain are limited to medical management, injection therapy and surgery. Medications to reduce pain are associated with significant morbidity and social concern. With 259 million opiate prescriptions, 2.1 million people suffer from substance abuse disorders, creating 1000 daily emergencies (5, 6). Opiate overdose death increased 200% from 2000 to 2016 (7). Health care providers face increasing burden and cost of chronic opiate use with increased scrutiny of monitoring patient usage and protecting against abuse.

Non-steroidal anti-inflammatory drugs (NSAIDs), are often the first line of treatment chosen by patients with chronic pain. NSAIDs are associated with gastrointestinal, hepatic, cardiovascular and anti-platelet complications, in part, due to patient overuse and concurrent use of prescribed and over-the-counter medications, producing 100,000 U.S. hospitalizations, 16,000 deaths and $2 billion in costs annually (8, 9). Steroid injections for short term pain relief can cause deleterious effects such as weight gain, exacerbating diabetes, cataracts, osteoporosis, and heighted risk of infections. Disease-Modifying

Anti-rheumatic Drugs (DMARDs), available in synthetic or biologic form, are associated with cancer, pneumonia, tuberculosis and death. Finally, surgery for chronic pain often has limited effect on pain and disability. Clearly, alternative approaches to relief of joint pain are needed.

Background on Amnionic Membrane: Amnionic membrane is clinically available from registered tissue banks globally. The form and methods for preparing amnionic membrane may vary affecting the specific contents and clinical results. Fresh amnion from healthy live births contains regenerative, anti-inflammatory, immunomodulatory and wound healing properties (10, 11). Amnionic membrane contains growth factors, cytokines, extracellular proteins and matrix metalloproteases inhibitors, including prostanoids, GDF-11, Wnt4, PGE2, IL-10, IL-1ra, HGF, VEGF, HGH, EGE, TGFâ, IL4,IGF-1and (TIMPs) (10, 12-18) that suppress cartilage damage (19, 20), stimulate endogenous chondrocyte proliferation for new cartilage (21), provide potent anti-inflammatory and anti-fibrotic effects in OA joint disease (22, 23). Of particular relevance to OA, PGE2 "reprograms" macrophages from the inflammatory M1 phenotype to the anti-inflammatory M2 phenotype (24). Amnionic membrane stimulates many metabolic processes including general protein and collagen synthesis, reducing pain, fibrosis, bacterial colonization and mediating tissue repair. Amnionic membrane is safe in humans and animals (25-29). Uses of amnionic membrane include conjunctival, pterygium, burn, chronic ulcer remodeling, as well as foot, ankle and orthopedic posterior lumbar surgery (11, 29).

Cryopreserved particulate amniotic membrane (CPAM) is cultured, particlized and cryopreserved amnionic membrane from placental tissue. Placental tissue donated by volunteers free of communicable disease undergoing caesarian section is processed to obtain amnionic membrane. The amnionic membrane is minimally manipulated under aseptic conditions, particlized and cryopreserved with DMSO, retaining much of its original matrix microstructure and cytokine profile. The cryopreserved amnionic membrane is used homologously as a protective barrier of membranous tissue placed over damaged joint tissue in patients with osteoarthritic joint pain. Patients receiving CPAM for joint pain have previously failed conservative and conventional therapies like pharmacological and physical therapy, with inadequate improvement of pain, making it medically necessary to proceed with interventional treatment. The standard procedure for therapeutic lumbar inter-laminar epidural injection with CPAM is as follows.

Background on Current Lumbar Inter-laminar Epidural Injection: After patient education and informed consent for treatment, the subject is placed into the prone position on a fluoroscopy table with moderate IV sedation. After confirming ID, site and side, the posterior lumbar region is widely prepped with Chloraprep, and sterile draped. The inter-laminar space at the level and side of the spinal lesion (confirmed by MRI and patient symptomatology) is identified with AP and oblique views fluoroscopically. The skin and underlying subcutaneous tissue are anesthetized with Lidocaine 1%. A 20 gauge, 3.5-inch Touhy needle is introduced and its direction and depth are confirmed with the AP and lateral fluoroscopic views, respectively. The needle is carefully advanced into the epidural space. Following needle placement 0.5 ml's of water-soluble contrast is injected to confirm needle position. Two 1 ml vials of CPAM at -90°C are gradually thawed at room temperature. After alcohol wipe, CPAM is drawn into a 10 cc sterile syringe containing 6 ml of sterile preservative free normal saline, injected over 30 seconds and cleared with 0.5 ml's saline. Post-procedure evaluation involves alertness, pain, stable vital signs (blood pressure, heart rate and oxygenation) and unchanged neurologic status at 15 , 30 and 60 minutes. After postoperative instructions, the patient is discharged when in stable condition.

Background on Ultrasound-Guided Knee Injection Utilizing The lateral Supra Patellar Approach: After patient education and consent for therapy, the patient is placed in the supine

position with the knee in extension. The lateral aspect is prepped with Betadine ×3 and sterile draped. The ultrasound probe is placed over the superior aspect of the patella to visualize the bony structures, followed by anesthetic ethyl chloride. One 1 ml vials of CPAM at -90°C is gradually thawed at room temperature. After alcohol wipe, CPAM is drawn into a 5 cc sterile syringe containing 4 ml of sterile preservative free normal saline. A 20-gauge 1.5 inch needle is directed into the suprapatellar bursa under ultrasound visualization, then the CPAM solution is injected under direct visualization. Post procedure evaluation involves alertness, pain, stable vital signs (blood pressure, heart rate and oxygenation) and unchanged neurologic status at 15 minutes, 30 minutes, and 60 minutes. After postoperative instructions, the patient is discharged when in stable condition. While CPAM injections in the knee and back are daily clinical practice at this institution, outcomes have not been previously reported.

Methods

To report the outcomes after injection of CPAM for joint pain, medical charts of 20 consenting adult subjects with joint pain, 10 back and 10 knee, previously treated with amnion at a single institution were reviewed for VAS pain scores, WOMAC daily activity function, opiate usage, and NSAID usage as well as for serious adverse events under an IRB-approved protocol.

Pain was evaluated using the Visual Analogue Scale (VAS) as assessed by the patient at baseline, 1 hour, 24 hours, 1 week, 2 weeks, 8 weeks and 12 weeks, consistent with the standard patient follow-up schedule at this institution.

Pain, stiffness and physical function were assessed using The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire at baseline, 2 weeks, 8 weeks and 12 weeks.

Presence of opiate and NSAID usage were recorded at baseline, 24 hours, 1 week, 2 weeks, 8 weeks and 12 weeks.

Statistical analysis was performed (SYSTAT 13) over time using a repeated measures analysis of variance. Missing data were estimated using multiple imputation for VAS and WOMAC, and "last value carried forward" for opiate and NSAID use. Location of injection, back or knee, was a covariate in the repeated measures analysis. A p-value of 0.05 was prospectively determined to represent significance.

Results

Medical records for 20 patients provided substantially complete data regarding demographics and outcomes. Mean age was 61.1 ±11.6 years (range 44-82). Of 10 back subjects, age was 65 ±14 years, range 46-81 years, 6 were males. Of 10 knees subjects, 8 left and 6 right, 4 were bilateral treatments, age was 57 ±6 years, range 44-65 years, 7 were male.

VAS-measured pain improved from 7.475 to 1.002 over 12 weeks (p<0.001) (Fig. 1). For back subjects, VAS-measured pain improved from 7.700 to 1.550. For knee subjects, VAS-measured pain improved from 7.250 to 0.553.

WOMAC-measured physical function scores improved from 46.0 to 10.8 over 12 weeks (p<0.001) (Fig. 2). For back subjects, WOMAC improved from 48.800 to 14.990. For knee subjects, WOMAC improved from 43.200 to 6.611.

Opiate use decreased from 55% to 15% over 12 weeks (p<0.001) (Fig. 3). For back subjects, opiate use deceased from 70% to 30%. For knee subjects, opiate use decreased from 40% to 0%.

NSAID use decreased from 80% to 10% over 12 weeks (p<0.001) (Fig. 4). For back subjects, NSAID use decreased from 60% to 10%. For knee subjects, NSAID use decreased from 100% to 0%. Location of injection was not a significant covariate factor for any outcome.

No serious adverse events were reported throughout 12 weeks. All four outcomes depict a consistent result with significant improvement. Extended follow-up averaged 360 days (179), range 122-601. Between 12 weeks and 6 months, one patient at 4 months reinjured his knee playing

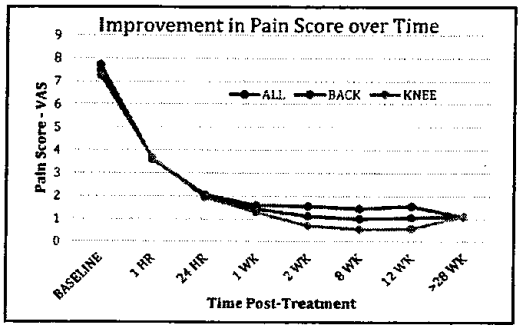

Fig. 1. Visual Analogue Scale (VAS) scores after treatment.

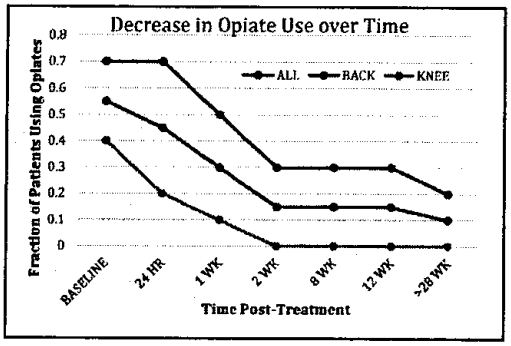

Fig. 3. Opiate use after treatment

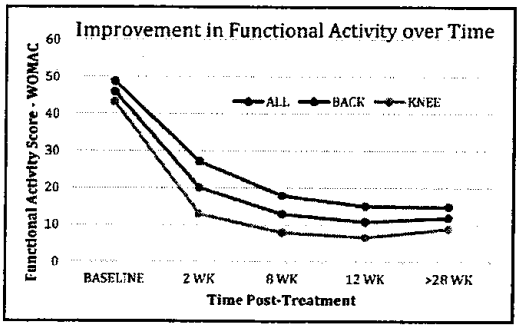

Fig. 2. The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores after treatment.

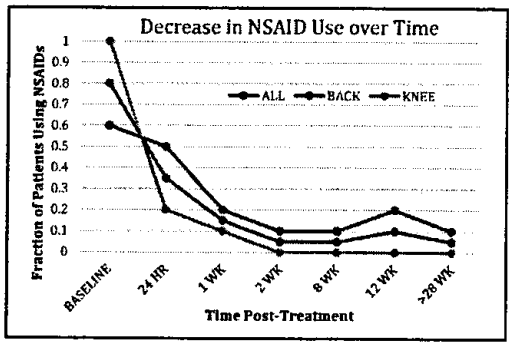

Fig. 4. NSAID use after treatment

tennis and was reinjected at 5 months. One patient at 6 months broke her toe walking her dog with temporary knee pain that resolved in 2 days without intervention. One patient with degenerative disc disease and lumbar spondylosis reported return of pain and was reinjected at 9 months. One patient with facet osteoarthritis and referred pain in the left lower extremity after 10 months requested another injection.Late interventions were without complication.

Discussion

Chart review of 20 patients with joint pain revealed the clinical benefits of injecting CPAM, whether back pain or knee pain. Improvement in pain, physical function, opiate use and NSAID use began promptly after treatment and was sustained over at least 12 week period, in most cases extending to 6 months or greater. Given that opiate use is CDC national epidemic secondary to dependency, overdose and abuse, leading to reluctance in prescribing and difficulty in managing patients on opiates, CPAM offers an important alternative (7).

Prior to treatment, patients were physically deconditioned secondary to their pain, complicating recovery and functional restoration. Therefore, patients were counseled on diet and gradual return to physical activity as critical adjunctive measures to achieving improvement in daily living metrics.

A dose of CPAM costs more than steroid; however, if pain relief from a CPAM injection

extends beyond 12 weeks, CPAM requires fewer procedures than steroids based upon Medicare utilization guidelines, e.g. 3 injections within a 6 month period. Since the costs of the interventional procedure and subsequent follow-up office visits are identical, it is plausible that the overall cost of patient management will be reduced with CPAM.

While this chart review supports the safety and effectiveness of CPAM, chart review has obvious limitations. For example, treatment was not prospectively randomized against a control depicting the current standard of care. Therefore, further research may provide additional confirmation regarding longer term outcome of pain relief, sustained functional activity, dependence on medications with known morbidity, and relative healthcare cost of treating these patients with advanced arthritic pain prior to joint replacement.

Conclusions

CPAM injection reduces pain, physical disability, opiate usage and NSAID usage in patients suffering from back and knee pain.

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