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Intradiscal Injection of an Autologous Alpha-2-Macroglobulin (A2M) Concentrate Alleviates Back Pain in FAC-Positive Patients

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Abstract

Objectives: A cartilage degradation product, the Fibronectin-Aggrecan complex (FAC), has been identified in patients with degenerative disc disease (DDD). Alpha-2-macroglobulin (A2M) can prevent the formation of the G3 domain of aggrecan, reducing the fibronectin-aggrecan G3 complex and therefore may be an efficacious treatment. The present study was designed to determine

a. The ability of autologous concentrated A2M to relieve back pain in patients with LBP from DDD and

b. The ability of FAC to predict the response to this biologic therapy.

Study design/setting: Prospective cohort

Patients: 24 patients with low back pain and MRI-concordant DDD

Main Outcome Measurements: Oswestry disability index (ODI) and visual analog scores (VAS) were noted at baseline and at 3- and 6-month follow-up. Primary outcome of clinical improvement was defined as patients with both a decrease in VAS of at least 3 points and ODI >20 points.

Methods: All patients underwent lavage for molecular discography and delayed FAC analysis and injection of platelet poor plasma rich in A2M (Cytonics Autologous Protease Inhibitor Concentrate, APIC) at the time of the procedure. ANOVA with Bonferonni correction for multiple comparisons was performed.

Results: Patients with FACT-positive assays were significantly more likely to show improvement in their VAS and ODI at follow-up. Mean VAS improvement in FACT-positive patients was 4.9 +/- 0.9 and 4.0 +/- 1.0 at 3 and 6-months, compared to 1.5 +/- 1.2 and 2.3 +/- 1.3 in those with negative FACT ($p < 0.0001$). Similarly, ODI improved on average 37 +/- 9.3 and 28 +/- 14 points at 3- and 6-months in FACT-positive patients compared to 9.4 +/- 11.9 and 12.6 +/- 11.8 points at 3- and 6-months in FACT-negative patients ($p<0.0001$).Correlation analysis demonstrated that a FACT-positive test correlates with improvement in 3-month VAS (Pearson $r = 0.83$; $p < 0.0001$) and ODI (Pearson $r = 0.71$; $p<0.0001$) and 6-month VAS (Pearson $r = 0.58$; $p<0.0001$) and ODI (Pearson $r = 0.53$; $p<0.0001$). When a 20-point ODI improvement cut-off is applied, 77% of FACT+ patients and 27% of FACT-negative patients meet this strict definition of clinical improvement.

Conclusion: The results of this investigation suggest that autologous A2M may be an efficacious biologic treatment in discogenic pain and that FAC may be an important biomarker in patient selection for this treatment. Patients who are "FACT+" within the disc are more likely to demonstrate clinical improvement following intradiscal autologous A2M injection. We utilized a definition of clinical improvement that was in excess of the minimal clinically important difference (MCID). Additionally, our defined outcome measure was a combination of two universally accepted outcome parameters (ODI and VAS). The current study provides evidence for a molecular biomarker that may improve patient selection and thus clinical outcomes in the treatment of discogenic back pain. Further study utilizing placebo-controlled trial is warranted.

Keywords: Biologic; Disc; Alpha 2 Macroglobulin; A2M; Back Pain; APIC; FAC; FACT

Abbreviations: FAC: Fibronectin-Aggrecan Complex; DDD: Degenerative Disc Disease; A2M: Alpha-2-Macroglobulin; ODI: Oswestry Disability Index; VAS: Visual Analog Scores; APIC: Autologous Protease Inhibitor Concentrate; MCID: Minimal Clinically important Difference; CLBP: Chronic Low Back Pain; MRI: Magnetic Resonance Imaging; HNP: Herniated Nucleus Pulposus; IRB: Institutional Review Board; ELISA: Enzyme-Linked Immune Sorbent Assay; OD: Optical Density; TMB: Tetra Methyl Benzidine; ANOVA: Analysis of Variance; ADAMTS: A Disintegrin-like And Metalloproteinase with Thrombo Spondin; MMPs: Matrix Metalloproteinase's; PDGF-BB: Platelet-Derived Growth Factor-BB; bFGF: basic Fibroblast Growth Factor; VEGF: Vascular Endothelial Growth Factor; TGF-B1: Transforming Growth Factor-Beta1

Introduction

Non-radiating chronic low back pain (CLBP) is a common patient complaint. A small percentage of individuals with persistent, or recurrent back pain report serious disabling symptoms, yet this small proportion of individuals result in a great burden to the health care system. Despite this, the etiology of chronic back pain associated with reported disability is often unknown. The intervertebral disc is a spinal structure that is sometimes labeled as the "pain generator" and the cause of CLBP when an alternate source is not identifiable. Degeneration of the disc occurs as part of the natural aging process and usually involves not just the disc, but also arthritic facet joints, ligamentous changes, bone remodeling, and reactive changes beneath the endplates.

Therefore, while a large proportion of the asymptomatic population will have abnormal appearing discs on imaging tests such as magnetic resonance imaging (MRI)[1] few have these findings as a strictly isolated pathology. This confounds attempts to identify the source of CLBP and can lead to unnecessary surgical or percutaneous interventions that target radiologically abnormal disc (s) as the source of CLBP. There is consequent controversy regarding how often the disc alone is the primary source of CLBP – so called "primary discogenic pain"; which connotes that the disc alone is responsible for all or nearly all of the patient's reported illness, irrespective of other spinal or regional pathology, social or psychological co-morbidities and other generalized pain syndromes.

Protein biomarkers associated with lumbar disc disease have been studied as diagnostic indicators and therapeutic targets. A cartilage degradation product, the Fibronectin-Aggrecan complex (FAC) identified in the epidural space, has been shown to predict response to lumbar epidural steroid injection in patients with radiculopathy from herniated nucleus pulposus (HNP) and identified in patients with degenerative disc disease (DDD) [2,3]. A therapeutic agent that prevents the formation of the G3 domain of aggrecan will reduce the fibronectin-aggrecan G3 complex and accordingly may be an efficacious treatment. Since the production of the G3 domain of aggrecan is catalyzed by different known classes of proteases, a common inhibitor of all of these proteases could be an ideal therapeutic agent. Such a protease inhibitor is found in plasma and synovial fluid, alpha-2-macroglobulin (A2M). The present study was designed to determine the ability of FAC to predict response to biologic therapy with concentrated autologous A2M for patients with LBP from DDD.

Methods

Subjects: Independent Institutional Review Board (IRB) approval was obtained (Sterling, Inc., Atlanta, GA, USA), and all patients provided informed consent for study participation. Patients considered candidates for intradiscal injection were 18 years of age or greater with a history of low back pain complaints primarily dictated by pain with associated sensory symptoms and/or low back pain for 6 months or more who had failed expectant management with NSAIDS, activity modification, and/or physical therapy. All patients had an MRI that demonstrated signs of degenerative disc disease at one or more levels with a Pfirrmann grade of II to IV. Patients ranging in age from 24 to 62 with axial back pain of at least 6 months duration were enrolled in this study.

The patients were identified among 55 consecutive patients offered study enrollment for the evaluation of their chronic pain. Patients were recruited from the private practice of a two board-certified and fellowship-trained orthopedic spine surgeons (PXM, GJS) during the period of May 2014 to November 2014. Patients with a history of oral or injected corticosteroid medication within a three month period prior to disc injection, those with chronic medical conditions associated with metabolic or inflammatory disorders (insulin-dependent diabetes mellitus, severe coronary artery disease, rheumatic or autoimmune diseases) were excluded from the study.

Sample acquisition, storage and preparation: All patients underwent lavage for molecular discography and delayed FAC analysis and injection of platelet poor plasma rich in A2M at the time of the procedure. A lavage for molecular discography was undertaken prior to injection as previously described [4,5]. Briefly, the patient was positioned prone on a radiolucent table, and monitored anesthesia was induced. After preparation with 1% povidone iodine, a 20 gauge spinal needle was placed into the disc space with the use of C-arm fluoroscopy in multiple planes.

Lavage was undertaken by injection/aspiration of approximately 1-2.5 cc of 0.9% normal saline without preservative by use of a 3 cc syringe. The lavage fluid was aliquoted into a sterile polypropylene tube and frozen at -80 ºC until the time of sample analysis [6]. At the time of analysis, each patient sample was thawed to room temperature, clarified by centrifugation at 5000g, and filtered using 0.45 µm low protein binding filter. The collected filtrate was immediately assayed as described below.

Sandwich Enzyme-linked immune sorbent assay (ELISA) analysis: This has been previously described [7]. A heterogeneous sandwich enzyme-linked immune sorbent assay (ELISA) was developed and validated with a previous series of patients [7]. This assay detects a protein complex offibronectin and the aggrecan G3 domain (i.e., FAC). A heterogeneous ELISA was developed for detection of FAC with the use of an antibody against one protein for capture and against the other protein for detection. Assay conditions were optimized to minimize background signal, and individual proteins were used as negative controls. In summary, an anti-aggrecan G3 domain antibody (Santa Cruz Biotechnology, Santa Cruz, CA) in phosphate-buffered saline/Tween 20/thimerosal was used to coat a 96-well micro plate.

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The plate was treated with bovine serum albumin in the same buffer overnight at 4°C to block excess binding sites, then washed with 6 washes of phosphate-buffered saline/ Tween 20/thimerosal. The centrifuged and filtered sample was aliquoted at 3 serial dilutions in triplicate into the microplate and incubated for 1 hour to facilitate binding of the complex to the immobilized antibody. After washing 6 times with the wash buffer, an anti fibronectin antibody labeled with horseradish peroxidase (US Biological, Swampscott, MA) was added and incubated for 1 hour. After 6 washes, the 3,3= ,5,5= -tetra methyl benzidine (TMB) substrate was added and the reaction product was measured by optical density (OD) at 450-nm wavelength (therefore measurements are reported in relative OD units rather than an absolute concentration). Human fibronectin (BD Biosciences, San Jose, CA) at 10 g/mL concentration was used as a negative control.

Clinical baseline and outcome measures: Oswestry disability index (ODI) and visual analog pain scores (VAS) were noted at baseline and at 3- and 6-month follow-up. Primary outcome of clinical improvement was defined as patients with both a decrease in VAS of at least 3 points and ODI >20 points.

Statistical analysis: Analysis of variance (ANOVA) with Bonferonni correction for multiple comparisons was performed using Aca Statand Stat Calc (Aca Stat Software, VA, and USA).

Results

Forty patients were enrolled in the study and underwent intradiscal injection with molecular discography. Of these, a complete data set with 6 months of follow up was available for 24 subjects – 11 females and 13 males with a median age of 47.5 (range 24-62). Patients with FACT-positive assays were significantly more likely to show improvement in their VAS and ODI at follow-up. Mean VAS improvement in FACT-positive patients was 4.9 +/- 0.9 and 4.0 +/- 1.0 at 3 and 6-months, compared to 1.5 +/- 1.2 and 2.3 +/- 1.3 in those with negative FACT ($p < 0.0001$).

Similarly, ODI improved on average 37 +/- 9.3 and 28 +/- 14 points at 3- and 6-months in FACT-positive patients compared to 9.4 +/- 11.9 and 12.6 +/- 11.8 points at 3- and 6-months in FACT-negative patients ($p<0.0001$). Correlation analysis demonstrated that a FACT-positive test correlates with improvement in 3-month VAS (Pearson $r = 0.83$; $p < 0.0001$) and ODI (Pearson $r = 0.71$; $p<0.0001$) and 6-month VAS (Pearson $r = 0.58$; $p<0.0001$) and ODI (Pearson $r = 0.53$; $p<0.0001$). When a 20-point ODI improvement cut-off is applied, 77% of FACT+ patients and 27% of FACT-negative patients meet this strict definition of clinical improvement.

Discussion

Protein biomarkers associated with lumbar disc disease have been studied as diagnostic indicators and therapeutic targets. A complex molecular and cellular cascade of disc degeneration continues to be investigated, which involves inflammatory mediators (e.g. cytokines, nitric oxide, and signal transduction pathways), structural proteins and their degradation fragments (e.g. fibronectin, aggrecan, and collagens), and proteases /protease inhibitors (e.g. matrix metalloproteinases, aggrecanases) [8-13].

Biomarker studies and the emergence of FAC as a diagnostic led us to hypothesize and test a potential new therapeutic method for treating DDD. In short, the biomarker we discovered and used for the diagnostic test is a complex of fibronectin and the G3 domain fragment from aggrecan [6,7], the most abundant protein in cartilage. The G3 domain fragment results from pathophysiological proteolytic activity in the joint and has been shown to activate the complement pathway leading to inflammation and the production of elastase and cathepsin-G [14,15].

We hypothesized that the reduction or elimination of these fragments would diminish the formation of the Fibronectin-aggrecan complex, and accordingly have a therapeutic effect. We hypothesized that alpha-2-macroglobulin (A2M), a general protease inhibitor present in blood at 0.1 – 6mg/ml, would be a good candidate for an inhibitor of this proteolytic activity. Upon further investigation, we found A2M in synovial fluid of a healthy person to be in the 10 – 30 ug/ml range, whereas in painful joints A2M is found to be in the range of 80 – 300ug/ml. This observation is consistent with the idea that A2M is a natural defense mechanism against proteolytic activities, and may be a very useful therapeutic agent to prevent or decelerate the progression of OA and other joint and spinal disc degenerative diseases.

A2M is a plasma glycoprotein that functions as a very effective protease inhibitor throughout different tissues and extracellular spaces. Cartilage degradation in OA and DDD has been associated with increased activity of several catabolic enzymes, including A Disintegrin-like And Metalloproteinase with Thrombo Spondin (ADAMTS)-4 and ADAMTS-5, matrix metalloproteinases (MMPs) [14,16,17], and inflammatory proteases (elastase and cathepsin-G) [18] as well as cytokines like TNF-alpha and IL-1-Beta, all of which are inhibited by A2M [19-21]. A2M is known to inhibit all of these proteases, though to different extents. Additionally, a recent independent investigation confirmed that A2M was the principle protease inhibitor for prevention of OA [19].

The present study has several limitations, the first being a small sample size, although a statistically-significant difference between the FACT-negative and FACT-positive groups in terms of patient-reported outcomes at both time points was observed. This study was of short-duration – with only 6-month follow up. Further study will be needed to determine whether this treatment has a long-term effect. If an injection can provide at least 6 months of pain relief, however, it may be clinically useful to help patients avoid or significantly prolong time to surgical intervention with disc replacement or lumbar spine fusion.

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Furthermore, this study utilized a proprietary (APIC, Cytonics Corp) FDA-approved* method for providing an autologous solution that is elevated in A2M concentration at approximately 6-8 times mean plasma level, but other biochemical are also concentrated and delivered with this treatment, such as platelet-derived growth factor-BB (PDGF-BB), PDGF-AB, basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and transforming growth factor-beta1 (TGF-B1). Therefore, we cannot with certainty ascribe all therapeutic effects to A2M alone – these additional growth factors may provide anabolic stimulus to the intervertebral disc and thus also provide therapeutic benefit.

Conclusion

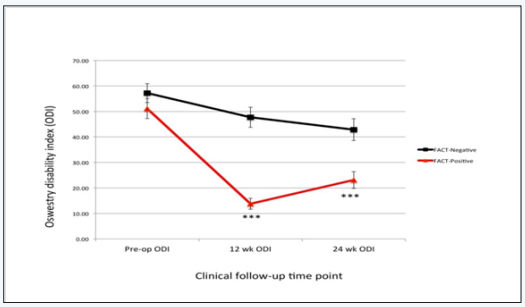

Figure 1: Clinical patient self-reported outcomes for pain (visual analog scale, VAS) before intradiscal injection of an autologous solution (APIC, Cytonics Corp) rich in A2M and at 3 months and 6 months post-injection. Square symbols represent the data for patients that tested negative to fibronectin-aggregan complex (FACT) at the time of injection ("molecular discography"), whereas the triangular symbols represent that data for patients that tested positive for the FACT. ***p<0.001. Error bars symbolize standard error of the mean.

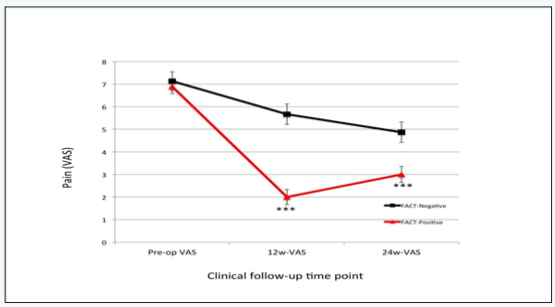

Figure 2: Clinical patient self-reported outcomes for function (Oswestry disability index, ODI) before intradiscal injection of an autologous solution (APIC, Cytonics Corp) rich in A2M and at 3 months and 6 months post-injection. Square symbols represent the data for patients that tested negative to fibronectin-aggregan complex (FACT) at the time of injection ("molecular discography"), whereas the triangular symbols represent that data for patients that tested positive for the FACT. ***p<0.001. Error bars symbolize standard error of the mean.

Patients who are "FACT+" within the intervertebral disc were more likely to demonstrate clinical improvement following an intradiscal injection of an autologous solution that is rich in A2M. The results of this investigation suggest that an autologous solution rich in A2M may be an efficacious biologic treatment for discogenic back pain. The current study also provides evidence that FAC is a molecular biomarker that may improve patient selection and thus clinical outcomes in the treatment of discogenic back pain (Figures 1 & 2).

*Cytonics APIC is FDA-approved for a similar application as platelet-rich plasma (PRP), which is for the enhancement of the manipulation properties of bone grafting. Therefore, this study discusses its use in a physician-directed manner that is not on-label with the FDA. Cytonics Corp. has recently completed the enrollment of a randomized controlled trial for the FDA on-label use of a similar APIC for mild-moderate osteoarthritis of the knee, but the approval is currently pending FDA review.

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