Evaluation of an Amniotic Suspension Allograft in a Rat Knee Pain Model

Kelly Kimmerling Ph.D.[1], and Katie C Mowry Ph.D.[1]
[1]Organogenesis Inc., 2641 Rocky Ridge Lane, Birmingham, AL 35216

Statement of Purpose

Osteoarthritis affects 30.8 million Americans[1]; this number will continue to grow as the population ages. IL-1 and TNF-α are two potent pro-inflammatory cytokines associated with OA[2]. Placental-derived membranes contain growth factors and anti-inflammatory cytokines[3], such as IL-1 receptor antagonist (IL-1Ra) and tissue inhibitors of metalloproteinases (TIMPs), that could help dampen the inflammatory cascade associated with OA.

The purpose of this study was to evaluate inflammation, pain, and function responses following intra-articular treatment with an amniotic suspension allograft (ASA) in a rat monosodium iodoacetate (MIA) knee pain model.

Methodology & Procedures

Male Lewis rats were injected with 2mg of MIA. 7 days following MIA injection, rats received an intra-articular injection of saline (vehicle control), ASA (25µL or 50µL), or triamcinolone (positive control). All injections were 50µL total volume, and each group consisted of n=10. Five rats were used as age-matched controls and did not undergo MIA injection or subsequent treatment. Rats were sacrificed at day 21; between days 7 and 21, rats underwent behavioral testing. These tests included Von Frey analysis (pain threshold using filaments), incapacitance testing (weight bearing measurement using force plates), and knee caliper measurements (swelling measurement). Serum and synovial fluid were collected at sacrifice for analysis using enzyme-linked immunosorbent assays (ELISAs) and a rat inflammatory panel. Animal behavioral data was analyzed using area under the curve with a one-way ANOVA and a Dunn's post-hoc test, while serum and synovial fluid data was analyzed using a one-way ANOVA and a Tukey post-hoc test.

Our hypothesis was that treatment of rats with ASA following MIA induction would result in reduced pain and inflammation and improved function.

Literature Review

Placental-derived membranes have a long history of use in wound care[4]; however, recent use in orthopaedics[5] has resulted in beneficial evidence supporting use in these applications. These membranes consist of the amnion and the chorion; each of these layers contain a number of growth factors and cytokines that could provide a benefit in inflammatory diseases[3]. Previously published pre-clinical studies have shown that the injection of placental-derived tissues are well tolerated and safe[6-7]. An injection of micronized amnion/chorion was retained in the joint space for 21 days, and resulted in reduced lesions, cartilage surface erosions, and proteoglycan loss in an MMT model of OA[6]. An injection of particulated amnion/umbilical cord resulted in increased cartilage thickness and volume and a reduction of lesion area over 4 weeks[7]. To date, only one clinical study has been published on the use of placental-derived membranes; the published pilot study[8] examined the use of an amniotic suspension allograft (ASA) in six patients with Kellgren-Lawrence grade 3 knee osteoarthritis. Patients reported a reduction in pain, improvement in function, and the injection was well-tolerated.

Behavioral Testing Results

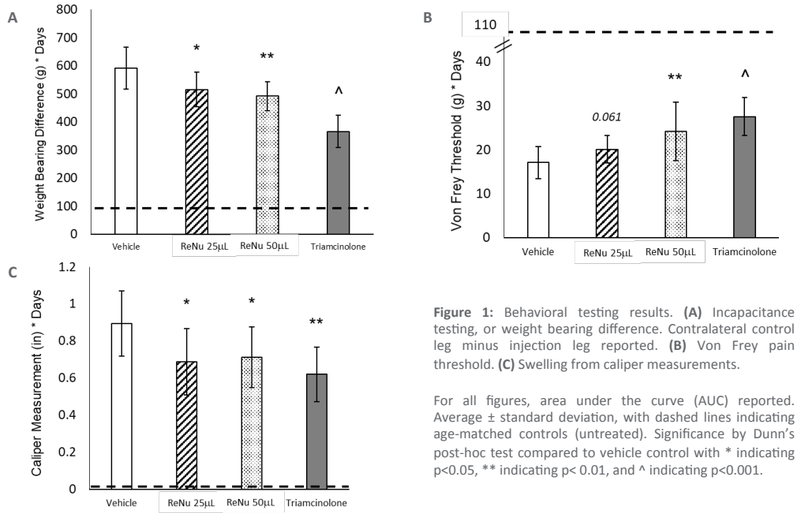

Figure 1: Behavioral testing results. **(A)** Incapacitance testing, or weight bearing difference. Contralateral control leg minus injection leg reported. **(B)** Von Frey pain threshold. **(C)** Swelling from caliper measurements.

For all figures, area under the curve (AUC) reported. Average ± standard deviation, with dashed lines indicating age-matched controls (untreated). Significance by Dunn's post-hoc test compared to vehicle control with * indicating p<0.05, ** indicating p< 0.01, and ^ indicating p<0.001.

Synovial Fluid Results

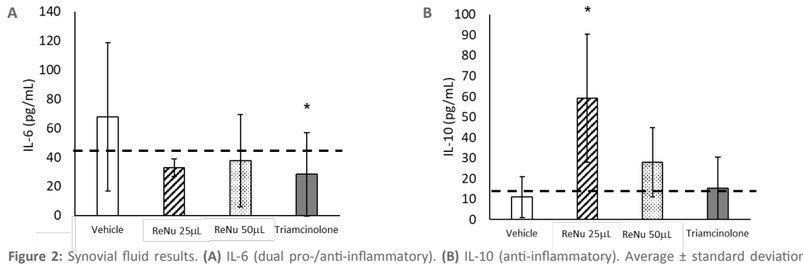

Figure 2: Synovial fluid results. **(A)** IL-6 (dual pro-/anti-inflammatory). **(B)** IL-10 (anti-inflammatory). Average ± standard deviation; dashed lines indicate age-matched controls. Significance by Tukey post-hoc test compared to vehicle with * indicating p<0.05.

Serum Results

Cytokine	Vehicle	ASA (25µL)	ASA (50µL)	Triamcinolone
IL-1β	59.36 ± 7.23	53.79 ± 3.24	57.68 ± 4.85	57.64 ± 5.90
IL-6	196.6 ± 21.53	204 ± 12.98	181.4 ± 42.2	197 ± 15.67
IL-10	209.2 ± 11.92	198.2 ± 27.1	219.6 ± 9.03	228.8 ± 9.12
MCP-1	1621.7 ± 398.53	1524.59 ± 283.41	1754.15 ± 460.05	1507.56 ± 326.85
TIMP-1	15133 ± 5231.15	13062 ± 3679.61	13069 ± 4946.56	13205 ± 4333.91
TNF-α	19.75 ± 0.67	19.52 ± 0.94	19.96 ± 0.67	20.04 ± 0.95

Table 1: Serum results. Average ± standard deviation reported for each group. No statistical significances between groups by Tukey post-hoc test.

Analysis & Discussion

Weight bearing difference between the treated and contralateral control limbs were significantly smaller in the ASA and triamcinolone groups compared to controls (p<0.05, p<0.01, p<0.001, Figure 1A). Rats treated with ASA and triamcinolone had significantly higher pain thresholds compared to saline (p<0.01, p<0.001, Figure 1B). Swelling as measured by knee calipers was significantly smaller in the ASA and triamcinolone groups compared to controls (p<0.05, p<0.05, p<0.01, Figure 1C). Levels of dual pro-/anti-inflammatory IL-6 after ASA treatment were decreased, but not significant compared to controls (Figure 2A); however, levels of anti-inflammatory cytokine IL-10 were significantly increased in the ASA group (p<0.05, Figure 2B). Finally, serum analysis showed no significant differences between groups (Table 1).

In this study, treatment with ASA improved function, pain thresholds, and decreased swelling. Furthermore, synovial fluid levels of IL-10 were significantly upregulated, promoting an anti-inflammatory environment in the joint space. In addition, there were no significant differences in groups following serum analysis; this shows that ASA treatment does not have a systemic effect and is localized to the joint space. This is the first study to examine pain and function following treatment with placental-derived products in a rat osteoarthritis pain model.

References

1. Cisternas MG, Murphy L, Sacks JJ, Solomon DH, Pasta DJ, Helmick CG: Alternative methods for defining osteoarthritis and the impact on estimating prevalence in a US population-based survey. Arthritis Care & Research 2016;68(5):574-580.
2. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nat Rev Rheumatol. 2011;7(1):33–42.
3. McQuilling JP, Vines JB, Kimmerling KA, Mowry KC. Proteomic Comparison of Amnion and Chorion and Evaluation of the Effects of Processing on Placental Membranes. Wounds a Compend Clin Res Pract. 2017 Jun;29(6):E38–42.
4. Trelford JD, Trelford-Sauder M. The amnion in surgery, past and present. Am J Obs Gynecol. 1979;134(7):833–45.
5. Riboh JC, Saltzman BM, Yanke AB, Cole BJ. Human Amniotic Membrane–Derived Products in Sports Medicine. Am J Sports Med. 2016;44(9):2425–34.
6. Willett NJ, Thote T, Lin AS, Moran S, Raji Y, Sridaran S, et al. Intra-articular injection of micronized dehydrated human amnion/chorion membrane attenuates osteoarthritis development. Arthritis Res Ther. 2014 Feb 6;16(1):R47.
7. Raines A, Shih M-S, Chua L, Su C, Tseng S, O'Connell J. Efficacy of particulate amniotic membrane and umbilical cord tissues in attenuating cartilage destruction in an osteoarthritis model. Tissue Eng Part A. 2017;23:12–9
8. Vines JB, Aliprantis AO, Gomoll AH, Farr J. Cryopreserved Amniotic Suspension for the Treatment of Knee Osteoarthritis. J Knee Surg. 2016;29(6):443–50.