Content Characterization of An Injectable Amniotic Suspension Allograft Available for Clinical Use

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BACKGROUND

- There is a growing body of literature providing proof-of-concept that amniotic and birth tissue products can provide effective benefits in orthopedic settings
- The mechanism of action and their contents are yet to be clearly elucidated
- The lack of information on the contents in amniotic-derived products has led to misperception in the field around actual levels of important proteins and cytokines present in these compounds

PURPOSE

The aim of this study is to characterize the protein contents of an injectable amniotic suspension allograft (ASA) currently in clinical development under an Investigational New Drug (IND) as a biological product for the treatment for knee osteoarthritis (OA)

HYPOTHESIS

- ASA contains varying concentrations of numerous growth factors, cytokines, chemokines, and proteins that are responsible for its mechanism of action

METHODS

Amniotic Suspension Allograft Preparation

- All ASA samples were created by one manufacturer (Organogenesis, Canton, MA).
- 10 total samples of ASA analyzed from five lots harvested from five fully independent human ASA donors undergoing scheduled c-section births of full-term healthy babies

Protein Content Identification

- A human multiplex array assay, human tissue inhibitor of metalloproteinase-3 (TIMP-3) enzyme-linked immunosorbent assay (ELISA) assay, and hyaluronic acid ELISA assay were used to evaluate the concentrations of regenerative factors, inflammatory-mediating cytokines, and proteases and inhibitors within each sample of the ASA
- Each of the five lots had two samples per donor

Human Multiplex Array Assay

- The human multiplex array was used to quantitatively measure 1000 preselected targets covering a multitude of the proteins of interest including IL-1ra (IRAP), IL-1B, IL-6, IL-8, , TGF-β, IGFBP, VEGF, PDGF, and various tissue inhibitors of metalloproteases (TIMPs)

TIMP-3 and Hyaluronic Acid Assays

- A specific TIMP-3 ELISA protein analysis was run at RayBiotech (Norcross, GA)
- A Purple-Jelley HA assay was performed in house

RESULTS

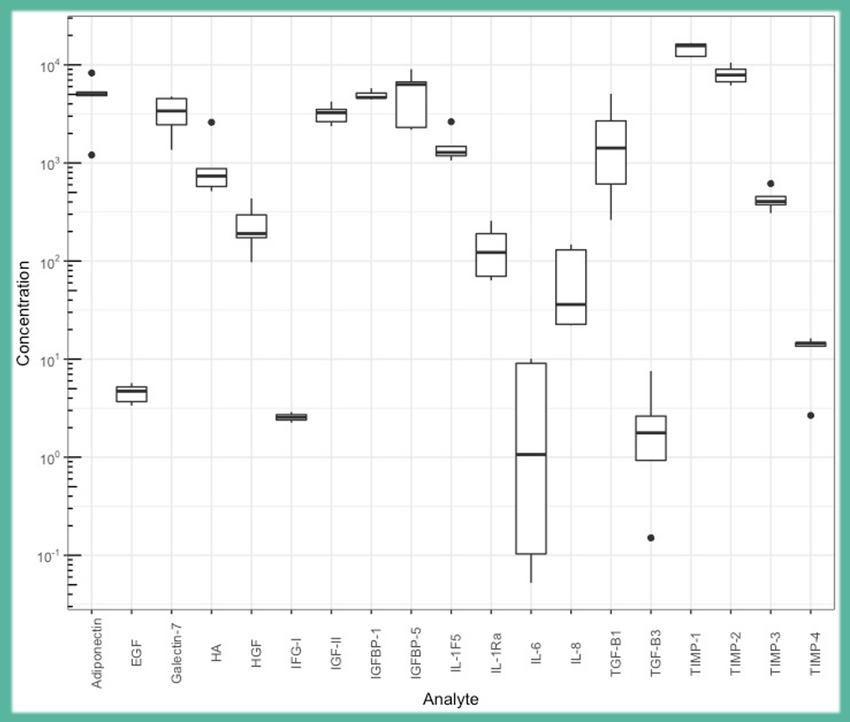

Figure 1. Boxplot of concentrations of analytes of interest from proteomic analysis. EGF = epidermal growth factor, HA = hyaluronic acid, HGF = hepatocyte growth factor , IGF = insulin-like growth factor, IGFBP = insulin like growth factor binding proteins, IL = interleukin, TGF = transforming growth factor, TIMP = tissue inhibitor of matrix metalloproteinases

Table 1. Concentrations of Analytes of Interest to Orthopedic Applications (pg/mL)

Analyte	Mean (pg/mL)	SD (pg/mL)	Range Lower Limit (pg/mL)	Range Upper Limit (pg/mL)
Adiponectin	4923.72	2509.66	1205.5	8274.7
EGF	4.53	0.99	3.4	5.7
Galectin-7	3299.34	1428.13	1359.7	4535.2
HGF	238.36	130.94	97.2	435.4
IGFBP-1	4929.88	544.23	4449.8	5779.7
IGFBP-5	5302.23	2981.20	2181.4	9029.0
IGF-I	1.03	1.42	0.0	2.9
IGF-II	3199.60	735.15	2370.3	4228.2
TGF- β1	1350.74	2161.41	0.0	5072.0
TGF- β3	2.25	3.09	0.0	7.6
TIMP-1	14571.66	2291.10	11990.7	16728.9
TIMP-2	8069.92	1767.86	6170.6	10546.5
TIMP-3	432.00	115.80	308.2	615.9
TIMP-4	12.35	5.50	2.7	16.3
IL-6	3.80	5.15	0.0	10.1
IL-8	71.61	61.76	22.1	147.4
IL-1F5	1525.94	636.20	1059.9	2631.2
IL-1ra (IRAP)	140.75	82.83	63.6	257.8
Hyaluronic Acid	1060.13 (ug/mL)	871.97 (ug/mL)	515.9 (ug/mL)	2599.63 (ug/mL)

Values calculated from sum of concentrations from supernatant and pellet for each sample by analyte.

RESULTS

- Of 1,002 proteins in the assays, 179 proteins demonstrated concentrations above minimum level of detections in all 10 samples
- Descriptive data on proteins of interest to orthopedic applications are in **Figure 1** and **Table 1**

- Regarding analytes pertinent to orthopedic applications, the five analytes pertinent to orthopedic applications highest in concentration included:
  - TIMP-1 (mean 14571.66 ± 2291 pg/mL)
  - TIMP-2 (mean 8069.92 ± 1767.86 pg/mL)
  - IGFBP-5 (mean 5302.23 ± 2981.20 pg/mL)
  - IGFBP-1 (mean 4929.88 ± 544.23 pg/mL)
  - adiponectin (mean 4923.72 ± 2509.65 pg/mL)

- Of the 19 analytes of interest, 15 were present in all donor samples
- TIMPs, HA, and IL-1Ra (IRAP) were detected in all the samples, whereas some samples did not contain IL-6, TGF-β1, TGF-β3, or IGF-1

- IL-1Ra (IRAP) was detected a mean of 140.75 ± 82.8 pg/mL with the concentration of IL-1B was below level of detection in all but one sample. In this sample, an IL-1ra:IL-1B ratio of 14.9 was calculated

- HA was detected in all samples and at a mean concentration of 1.060 ± 0.872 ug/mL.

CONCLUSION

- Proteomic analysis demonstrated ASA shared the same regenerative agents (e.g. IGFBP-1, IGFBP-5, TGF-β1, TGF-β3) and immune-modulating signaling agents (e.g. IL-6, IL-8, TIMP-1, TIMP-2, TIMP-3, TIMP-4) present in fresh amnion but did not share the angiogenic agents (e.g. VEGF, PDGF)

- The contents of ASA shared many similarities to other injectable orthobiologics including BMAC and PRP such as TGF-β1, IL-1ra (IRAP), IL-8, and IL-1B

- IL-1ra (IRAP) was present in all 10 samples and the ratio of IL-1ra:IL-1B was calculated at 14.9 in the one sample with IL-1B above the minimum level of accurate detection. This is important as an IL-1ra: IL-1B ratio of 10:1 to 100:1 has been suggested to provide effective therapy and sufficiently block IL-1.

- HA was found at concentrations 6.25%-15% of the concentrations seen in standard orthopedic injections

- Future studies will need to determine which individual factor or combination of factors are responsible for the clinical benefit ASA demonstrates in the setting of knee OA and if there are any individual or synergistic threshold concentrations necessary for clinical effects to be seen