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# A method to induce Interleukin-1 Receptor Antagonist Protein from autologous whole blood

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ABSTRACT

*Objective:* Current orthopedic therapies, aimed solely at symptomatic control, are unable to restore the cytokine imbalance that produces the hallmark clinical profile of osteoarthritis. While a myriad of chemical factors in the cytokine network stimulate local joint inflammation and pain, Interleukin 1 (IL-1) is widely recognized as a key offender and a potential therapeutic target. The purpose of this article is to describe a novel, on-site, point of service process (Arthrokinex™) to induce Interleukin 1 Receptor Antagonist Protein (IL-1-Ra or IRAP) from whole blood aimed at inhibiting the destructive intra-articular effects of IL-1.

*Methods:* 53 patient charts were included in this retrospective chart review study. Venous blood from the selected participants had been harvested and centrifuged to isolate Platelet Rich Plasma and Platelet Poor Plasma. These layers were extracted and incubated for 30 min in a specialized syringe containing medical grade concentrator beads. After centrifuge filtration, the supernatant containing IL-1-Ra was extracted. Anti-inflammatory (IL-1-Ra, IL-10) and pro-inflammatory (TNF α, IL-1 β) cytokines of baseline whole blood were compared to the conditioned serum following quantification using ELISA.

*Results:* On average, a 32-fold increase (baseline, 550 pg/mL; post conditioning 17,537 pg/mL) in IL-1-Ra concentration was observed after the brief interaction of blood with the concentrator bead surface. IL-1-Ra, if present in concentrations that are 10–100 times higher than IL-1β, will block the interaction of IL-1β with cell surface receptors. At these increased concentrations, Arthrokinex™ induced IL-1-Ra joint injections produce an IL-1-Ra to IL-1β ratio of 999:1. Post conditioning levels of IL-1β and TNF α were not clinically significant.

*Conclusion:* The Arthrokinex™ blood conditioning process has the ability to rapidly induce IL-1-Ra without increasing the pro-inflammatory cytokine profile.



## 1. Introduction

The poorly understood, multi-factorial pathogenesis of osteoarthritis (OA) provides a significant challenge to treat the estimated 27 million people in the US [1] affected by the progressively debilitating disease. Articular cartilage destruction, subchondral bone remodeling and synovitis are the chief causes of the clinical manifestation of OA, which include pain, swelling, and stiffness of the affected joint. Axiomatically, these symptoms can pose a dramatic hindrance on daily activities depending on severity. Analgesic drugs, nonsteroidal anti-inflammatory drugs (NSAIDs) and intra-articular (IA) corticosteroid injections are the currently recommended pharmacologic interventions for knee OA [2]. Given the limited scope and effectiveness of these treatment options aimed solely at symptomatic control of the disease, many patients are forced to undergo surgery. Regenerative therapies including platelet rich plasma and mesenchymal stem cells, are on the rise despite conflicting evidence of supportive data. Early data indicated the potential musculoskeletal benefits and cost effectiveness of platelet rich plasma (PRP) injections. The majority of trials have failed to provide evidence for the increased use of PRP therapy [3]; however it is difficult to pinpoint if this is due to the actual treatment regimen or the lack of standardized protocols, platelet separation techniques and outcome measures. Interestingly, a recent systemic review and meta-analysis reported PRP IA injections are significantly superior to placebo and hyaluronic acid for the treatment of knee OA (all other outcomes were excluded) [4]. Despite inconsistent results, the market value of

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PRP is estimated to reach $126 million by 2016 [5]. A number of recent studies are beginning to emerge that reveal the potential for autologous adipose tissue derived mesenchymal stem cells, albeit to a much smaller degree than PRP, for the treatment of OA [6]. The need for an out-patient, on-site, point of service, low cost symptom relieving and possibly chondroprotective drug is evident given the already high prevalence of arthritis in the US, which is expected to increase to nearly 67 million people by the year 2030 [7], the significant financial burden to the patient ($703/year) and insurer ($3080/year) [8] and the lack of effective non-surgical options.

A recently improved knowledge of the molecular mechanisms underlying this disease has led to the exploration of biotherapeutic strategies. One approach that holds promise is the inhibition of interleukin-1β (IL-1 β), a major cytokine promoting the catabolic activity associated with OA affected joints [9]. Attur et al. [10] reported the presence of biologically active IL-1β in OA-damaged cartilage providing the rationale to explore blockade of this molecule as a target to facilitate cartilage repair and potentially reverse degradation. Different methods to specifically inhibit Interleukin 1 (IL-1) have been tested. Briefly, those include the application of soluble IL-1 receptors, monoclonal antibodies against IL-1 or IL-1 receptor 1, blocking the formation of active IL-1β, gene therapy, and the application of IL-1 receptor antagonist protein (IL-1-Ra) [9], which serves as the focus of this investigation. It is unclear which method is most effective; however, the success of three commercially available IL-1-Ra products (recombinant Anakinra™, autologous Orthokine™ and Arthrex™) led to the development of our novel IL-1-Ra formulation process. The primary purpose of this investigation was to test our hypothesis that our on-site, point of service, minimal manipulation processing of whole blood would induce sufficient IL-1-Ra levels and IL-1-Ra:IL-1β block ratios. Secondary outcomes were twofold: (1) to ensure the Arthrokinex™ process did not increase the concentration of anti-inflammatory cytokines (TNF-α and IL-1β) and (2) to evaluate cytokines levels in the serum samples after being stored at −20 °C for at least one year.

## 2. Materials and methods

### 2.1. Study design

This was a retrospective chart review/proof of concept investigation aimed to quantify the ability of the Arthrokinex™ process to enhance IL-1-Ra in whole blood. All aspects of the study protocol were extensively reviewed and approved by IntegReview Institutional Review Board (IRB) as being considered exempt from requiring IRB approval as it met all requirements outlined in 45 CFR 46.101(b)(4), specifically (1) the research involves only the collection or study of pre-existing data, documents, records, pathologic specimens or diagnostic specimens and (2) the information will be recorded in such a manner that the subjects cannot be identified, directly or through identifiers linked to the subjects.

### 2.2. Participants

Existing charts were reviewed and a total of fifty three (53) patient charts met all inclusion criteria for this analysis: age >21 years, chronic OA for at least 3 months, patients were diagnosed with OA according to the American College of Rheumatology (ACR) criteria, radiographic evidence of OA and ⩾4 pain grade (on a numeric scale of 1–10). Exclusion criteria included: patient charts of those in generally poor health, pregnant or breast feeding, drug dependent (chronic opioid use, alcohol, etc.), undergone surgery or treatment of the affected joint within the last 3 months, lacked the mental ability to understand the treatment plan, systemic disease of the musculoskeletal system, bone cancer, metastasis or tumor-like lesions in the immediate proximity to the treated joint, fracture in the last 3 months, acute bacterial infection, blood clotting disorders, major psychiatric disease requiring therapy, and continuous corticoid or NSAID therapy due to other diseases. Informed consent was obtained from each participant and all work was performed in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

### 2.3. Processing of whole blood (Arthrokinex™)

Using aseptic techniques, 60 mL whole blood from the median cubital vein of fifty three (53) participants was harvested into a sterile 60 mL syringe containing 3 mL of anticoagulant citrate dextrose (ACD) solution and centrifuged (3200 rpm, 15 min). The resultant Platelet Rich Plasma (PRP) and Platelet Poor Plasma (PPP) were then extracted and the remaining layers, containing buffy coat and erythrocytes, were discarded. Both the PRP and PPP were transferred to a specialized, closed-system, centrifuge tube containing medical grade concentrator beads, mixed and allowed to incubate for 30 min at ambient temperature. After the short incubation period, centrifuge filtration (2000 rpm, 3 1/2 min) through a sterile 0.45 μm filter was completed and the resulting sterile filtrate was slowly drawn into 1 mL syringes. The 1 mL syringes could be used immediately for intra-articular injection or stored at −20 °C for future use.

### 2.4. Biomarker assays

The primary outcome of measuring IL-1-Ra (pre- and post-conditioning) was achieved by using the highly sensitive, commercially available quantitative sandwich enzyme-linked immunoassay technique (R&D Systems, Quantikine ELISA; Minneapolis, MN, USA. The manufacturer reports this kit, when run in accordance with standard Quantikine protocols, to be extremely sensitive (minimum detectable dose ranged from 2.2 to 18.3 pg/mL), specific (no significant cross-reactivity or interference was observed), precise (intra- and inter-assay CVs were 3.7% and 6.7%) and linear (all diluted samples fell with the dynamic range of the assay). Since sample concentrations were expected to fall outside the range of provided standards, serum was diluted 100 fold by adding 5 μl of sample to 495 μl of calibrator diluent. Resulting concentrations were calculated by subtracting the average zero standard optical density and log transforming IL-1-Ra concentrations versus the log of the optic density on a linear scale, and the best fit line determined by regression analysis. IL-1-Ra concentrations were only accepted if the standard curve correlation coefficient (r) reached 0.99 and the CV of each sample was under 20%.

As a secondary outcome, serum levels (pre- and post-conditioning) of pro-inflammatory (TNFα, IL-1β) cytokines and another anti-inflammatory cytokine (IL-10) were measured separately using ELISA. All kits reported comparable sensitivity, specificity, precision and linearity as described above. Similar to IL-1-Ra, all kits were run in accordance with standard Quantikine protocols.

### 2.5. Statistical analysis

SPSS 12.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Wilcoxon signed rank test were performed to analyze the statistical difference between baseline and post-processing cytokine levels. All results shown are the mean ± SEM of two or more experiments.