**NOVEL ANTI-INFLAMMATORY/CATABOLIC AND REGENERATIVE SECOND GENERATION PRP TREATMENT FOR KNEE OSTEOARTHRITIS.**

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**CONCLUSIONS:**

- Incubated blood is a source of anti-inflammatory/catabolic and regenerative agents.
- Incubation process leads to increasing synthesis of catabolic MMP 9 that could be successfully blocked by adding citric acid in certain concentration.
- The present in vitro study and clinical trial demonstrated that new second generation PRP ABP has a promising therapeutic effect for the treatment of knee OA.

**METHODS**

Blood samples from healthy donors were incubated using different techniques for 24h and analyzed for the presence of anti-inflammatory (IL-1ra), anti-catabolic (tissue inhibitors of metalloproteinases, TIMPs), regenerative (PDGF, TGF-β), pro-inflammatory (TNF-α, IL-1) and catabolic (matrix metalloproteinases, MMPs) molecules. Double-blinded controlled clinical study was conducted to evaluate clinical effectiveness and safety of the final product comprised from anti-inflammatory/catabolic and regenerative components using VAS and WOMAC scales.

**INTRODUCTION**

Osteoarthritis (OA) is degenerative joint disease characterized by cartilage damage and synovial inflammation. Autologous blood-derived products (PRP, Orthokine) target special inflammatory molecular pathways and have a beneficial therapeutic effects for inflammatory pathologies. This study assesses the in vitro and in vivo anti-inflammatory/catabolic and regenerative potential for OA treatment of a novel second generation PRP autologous blood product (ABP).

**DOUBLE-BLIND CONTROLLED CLINICAL TRIAL**

23 patients were recruited voluntarily. No study-related adverse events were reported. One patient didn’t complete follow up process due to no study related AE.

At the baseline visit, subjects meeting eligibility requirements were randomized 1:1 to either ABP or PRP (control) treatment arm. The scheduled treatment regimen was 1 dose per week for 4 weeks as an intraarticular injection into the affected knee. The following variables were created: dVASi (Difference in VAS pain score at baseline and at day i), dPAINi (Difference in WOMAC pain score at baseline and at day i), dSTIFFi (Difference in WOMAC stiffness score at baseline and at day i), dDAi (Difference in scores of WOMAC difficulty performing daily activities at baseline and at day i).

**BLOOD INCUBATION PROCESS LEADS TO INCREASING OF MMP9 CONCENTRATION**

Optimal blood incubation conditions for IL1-β production fail to stimulate production of catabolic MMP9 that could be reversed by Sodium Citrate (SC) addition.

**SC PREVENTS PRO-INFLAMMATORY CYTOKINES ENRICHMENT IN DOSE-DEPENDENT MANNER**

Human serum samples have been analyzed before incubation (1), after incubation at 37°C for 24h in the presence of saline (2) and SC (3), PRP (4) and in the final product comprised a serum with and without SC with and without SC (5, 6). Dose dependent SC concentration effect on IL-1β and TNF-alpha production. SC doesn’t affect anti-inflammatory/catabolic and regenerative agents enrichment.

**PRODUCTION OF ANTI-INFLAMMATORY/ CATABOLIC MOLECULES BY INCUBATED HUMAN BLOOD CELLS**

IL-1ra production is significantly stimulated by glass internal surfaces. IL-1ra levels in the final product increased in the presence and absence of agitation (3). A component of the IL-1ra concentration in human serum samples incubated in glass (4), with agitation, serum 2-3x higher than in saline 1:1 versus PRP 3-7x higher saline 4-4x, 12% saline vs 1x saline. IL-1ra levels (5) increased in saline to 180% concentrations after 24h (6) and other incubation at 24h for OA. Incubation process leads to significant decrease of IL-1β serum concentration.

**SC DOESN’T AFFECT ANTI-INFLAMMATORY/ CATABOLIC AND REGENERATIVE AGENTS ENRICHMENT**

Protein concentration levels of IL-1ra, PDGF, TIMP1 and TIMP2 in human serum samples before incubation (1), after incubation at 37°C for 24h in the presence of saline (2), SC (3), PRP (4) and in the final product comprised sera with and without SC with and without SC (5, 6). SC doesn’t affect IL-1ra and TNF-alpha production but in addition to a control saline for SC.

**CONCLUSIONS:**

- Incubated blood is a source of anti-inflammatory/catabolic and regenerative agents.
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- The present in vitro study and clinical trial demonstrated that new second generation PRP ABP has a promising therapeutic effect for the treatment of knee OA.