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

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INTRODUCTION

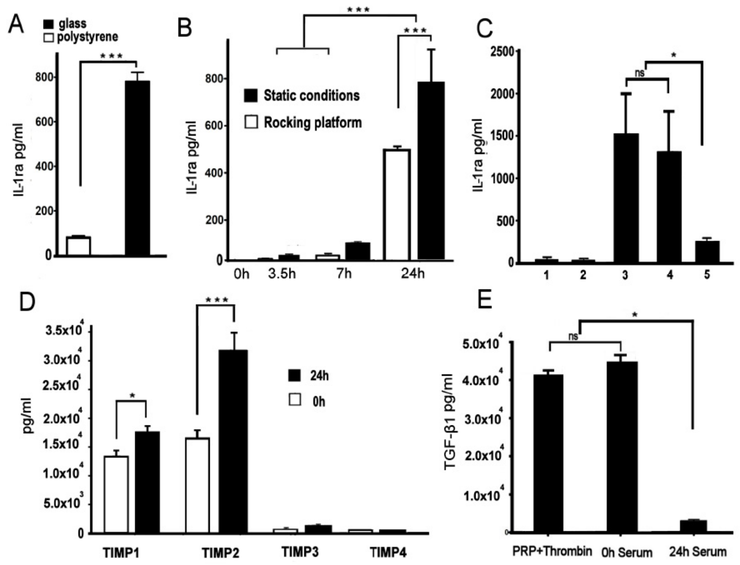
Osteoarthritis (OA) is degenerative joint disease characterized by cartilage damage and synovial inflammation. Autologous blood-derived products (PRP, Orthokine) target special inflammatory molecular pathways and have a beneficial therapeutic effects for inflammatory pathologies. This study assesses 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).

METHODS

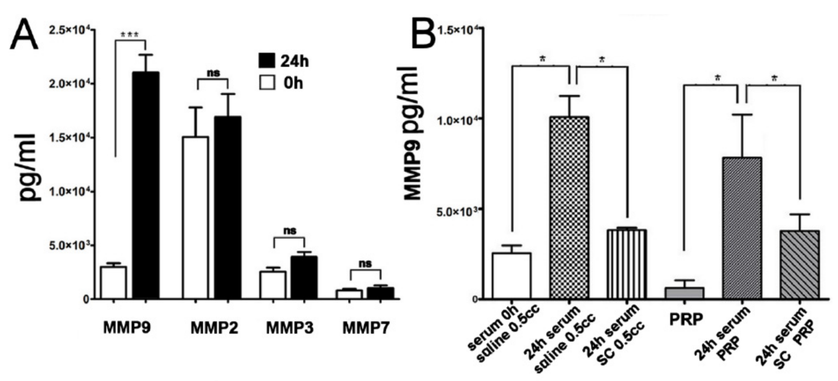
Blood samples from healthy donors were incubated using different techniques for 24h and analyzed for the presence of anti-inflammatory (IL-1ra), anti-catabolic (tissue inhibitors of metalloproteinases, TIMPs), regenerative (PDGF, TGF- β), pro-inflammatory (TNF-α, IL-1) and catabolic (matrix metalloproteinases, 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.

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

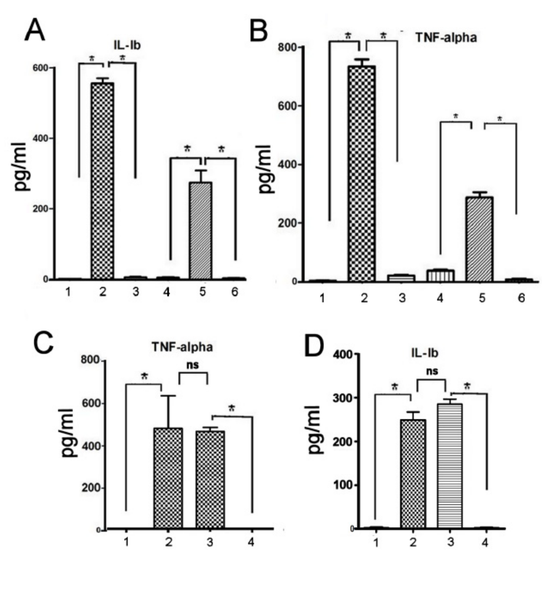
(A). IL-1ra production is significantly stimulated by glass internal surfaces, (B) A comparison of the IL-1ra levels in the human serum samples that were incubated for different lengths of time in the presence and absence of agitation (C). A comparison of the IL-1ra concentration in human serum samples incubated in glass tubes: **1-**0h incubation, serum **2-** 0h incubation 1:1 serum: PBS++, **3-** 24h incubated serum, **4-** 24h 1:5 diluted serum ,**5-** 24h 1:1 diluted serum (D) Analysis of TIMPs concentrations before (baseline level) and after incubation at 37ºC for 24h. (E) Incubation process leads to significant decrease of TGF-β1 serum concentration.



BLOOD INCUBATION PROCESS LEADS TO INCREASING OF MMP9 CONCENTRATION

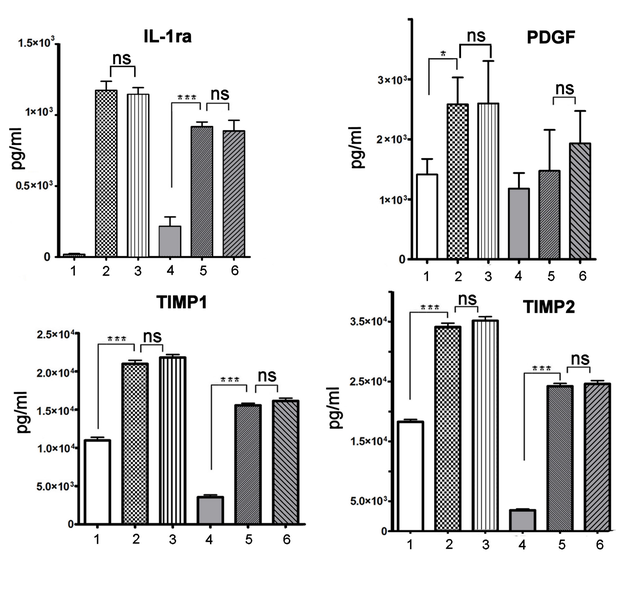


Optimal blood incubation conditions for IL1-ra production facilitate synthesis of catabolic MMP9 that could be reversed by Sodium Citrate (SC) addition.



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 as a final product contained a serum with and without SC with and without SC (5,6). (C,D) Dose depend SC concertation effect on IL-1b and TNF-alpha production Saline is added as a control substance for SC.

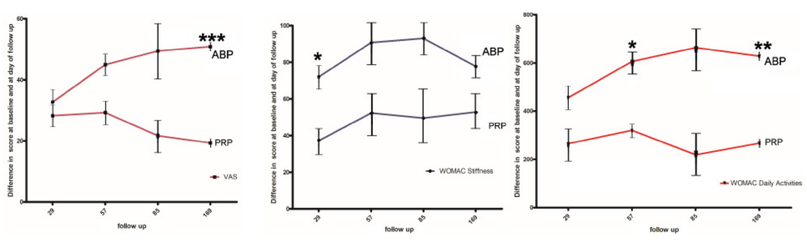


SC DOESN'T AFFECT ANTI-INFLAMMATORY/ CATABOLIC AND REGENERATIVE AGENTS ENRICHMENT

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 as a control (2) and SC (3), PRP (4) and in the final product contained a serum with and without SC with (5) and without SC (6).

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).



Except for difference in scores of WOMAC difficulty performing daily activities at baseline and at day 85, and day169 for PRP the measures for VAS, PAIN, STIFF, and DA for both treatments at baseline and at follow up days were all statistically significantly different. The improvement in the ABP arm was statistically significantly higher than the improvement in the PRP arm, in terms of dSTIFF29, dDA57, dVAS169, and dDA169.

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 in the treatment of knee OA.