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Signaling Proteins (Growth Factors and Cytokines) in Orthopaedics. Comparison of two blood processing techniques: ORTHOKINE® and ACP®.

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Summary:
ORTHOKINE[1], PRP[2], ACP[3] are successful blood-processing based autologous biological treatment options in orthopaedics. Platelet-Rich Plasma contains platelets ≥3x over baseline. ACP contains <2 fold platelet increase. Both work by mechanical blood separation. Differently, ORTHOKINE is based on incubation of whole blood and yields elevated Signaling Proteins (SP). ORTHOKINE outperforms ACP in SP content and quality.

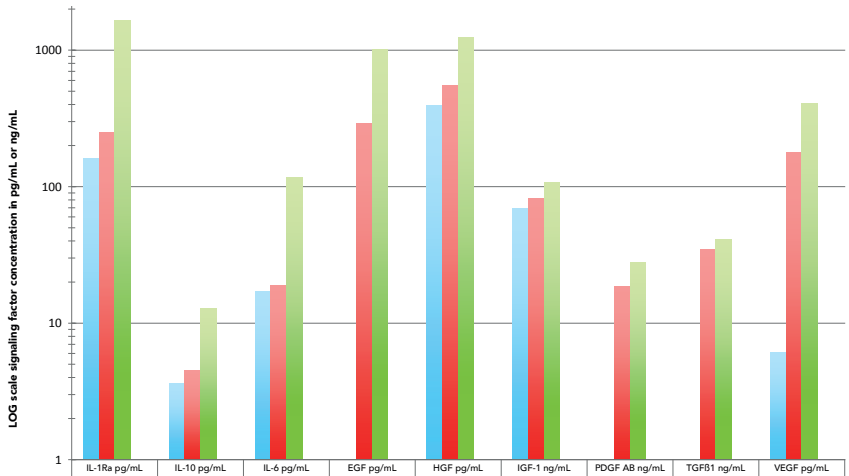
Introduction:
Autologous blood preparations for orthopaedic SP therapy are popular. Platelet-rich plasma (PRP) has become popular and is slowly starting to present RCT Level 1 proof of evidence. However, many different PRP techniques are used making comparison of clinical results difficult. The SP contained in PRPs include cytokines and growth factors (GF) considered helpful once SP are released by coagulation in situ.
Two non-PRP techniques were compared: ORTHOKINE-technique producing Autologous Conditioned Serum (ACS) and ACP plasma-separation technique producing autologous plasma (AP).
ORTHOKINE bases on accumulation of SP from all blood cells in the supernatant serum through incubation at body temperature of coagulating venous whole blood in specially taylored syringes (conditioning of serum). Conditioned cell free serum is injected intra-articularly, peri- and intra-tendinous and radicular.
ACP platelet-separation technique bases on physical separation of plasma from red and white blood cells (WBC) from anticoagulated venous blood. ACP-AP is injected in similar indications as ORTHOKINE-ACS.

Patients and Methods:
Blood from 9 volunteers was collected with ORTHOKINE (10mL) and ACP (12mL) devices. Blood was cell counted and processed according to the respective manufacturers' instructions. ORTHOKINE-ACS: blood was incubated 6 h, then centrifuged at about 3000g, separating serum from coagulum. ACP-AP was prepared by slow centrifugation of anticoagulated whole blood in ACP device. Supernatant plasma was collected for measurements. Blood cell counts were performed externally on ACP-AP and whole blood. ORTHOKINE-ACS contains no cells. All samples were stored at ≤-20°C until SP ELISA-measurement. Cells in ACP samples were lysed to make all SP content accessible for ELISA testing. ELISAs for IL-1Ra, IL-10, IL-6, EGF, HGF, IGF-1, PDGF-AB, TGFß-1, VEGF were performed with R&D Systems Kits within 1 week after sampling.
Results: Mean volume yield for AP: 3.4mL; for ACS: 3.7mL (Table1). SP concentration in ORTHOKINE-ACS: all 1.3-6.6x over ACP-AP (Figure1). Cytokine concentrations in ORTHOKINE-ACS: all 2-6.6x over ACP-AP (Figure1). AP contained mean platelet count 1.9x over baseline. Mean yield of platelets in AP: 85% (Table1). Highest differences: ACS over AP: IL-1Ra: 6.6x.

	ACP (10mL)	ORTHOKINE (10mL)	Typical PRP system
initial blood volume [mL]	12 mL (10,8 + 1,2 Citr)	10 mL	10-60mL
Final Volume [mL]	3,4 ±0,45	3,7 ±0,37	1-8 mL
Total Platelet Yield	1,6 x 10^9 (85%)	1,8 x 10^9 (100%)*	approx. 40-90%
Processing Time	15 min	20 min**	approx. 20-35 min
Storage possible?	n.d.	Yes, 7 months (≤20°C)	n.d.
Lot No	11027682	1108021-F0005	-

Table 1: Baseline Data for ORTHOKINE, ACP and typical PRP systems. *ORTHOKINE uses whole blood containing all cellular and a-cellular components. **Processing time plus 6 h incubation at body temperature.

Concentration of selected effector proteins at baseline, in ACP and in ORTHOKINE [pg/mL] and [ng/mL]



	IL-1Ra pg/mL	IL-10 pg/mL	IL-6 pg/mL	EGF pg/mL	HGF pg/mL	IGF-1 ng/mL	PDGF AB ng/mL	TGFß1 ng/mL	VEGF pg/mL
Baseline	161,4	3,6	17,1	0,1	396	69,2	0	1	6,1
ACP	250,3	4,5	18,9	289,2	550,1	81,9	18,6	34,5	177,8
ORTHOKINE	1642,7	12,7	117,7	1019,2	1238,3	107,1	28,1	41	409,5
ORTHOKINE/ACP	6,6	2,8	6,2	3,5	2,3	1,3	1,5	1,2	2,3

Discussion/Conclusions:
ORTHOKINE-ACS and ACP-AP provide SP considered helpful to support injured [cartilage] tissue. Here ACS has higher SP content than AP. GF content is largely dependent on platelet degranulation, but white blood cells can substantially contribute to GF and cytokine production. ACS gains from 100% of platelets and 100% of WBC. AP lacks ±15% platelets and virtually all WBC. Lower GF concentrations detected in AP are possibly attributable to this. Additionally, GF may partly derive from white blood cells (e.g. EGF and VEGF) while IGF-1 is present in high baseline concentrations in the circulation (±70ng/mL). Apparently there are GF reservoirs in WBC being liberated after ACS blood processing. Arguably, a higher SF dose may be predictive for better clinical effect. The high IL-1Ra content may explain the additionall superior anti-inflammatory action of ORTHOKINE-ACS[1]. Comparison of both approaches in experimental and clinical settings should provide more insight.

[1] Wehling et al. Biodrugs2007; 21(5): 323-332
[2] Nguyen et al. PM&R2011; Vol.3, 226-250
[3] Schippinger at al. JSSM2011; 10, 439-444

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