Fibrinogen-coated albumin spheres to accelerate bone healing in older rats

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DISCLOSURES: Study was supported by NIH 1R43AG072919-01A1. S-H. Park (N); I. Kovanecz (N); E. Ebramzadeh (N); R. Yen (N)

INTRODUCTION: Fracture healing results from a complex, sequential cascade of cellular events. Although the exact regulatory mechanisms for fracture healing are not yet fully understood, neo-vascularity as well as osteoblast recruitment at the callus site appear to play key roles in successful healing. We investigated a novel intravenous injectable biological product based on Fibrinogen-coated Albumin nano-Spheres (FAS). Extensive preliminary studies demonstrated multi-valent properties of FAS, including the ability to support and accelerate wound healing of multiple ulcerated-necrotic tissues, by promoting the mobilization of different lineages of progenitor cells, particularly Endothelial Progenitor Cells (EPCs), and decreasing levels of pro-inflammatory cytokines (including IL-6) in the blood. One of the main factors that affect bone healing in the elderly is the decline in the number and function of skeletal stem cells, which are essential for bone regeneration. We hypothesized that FAS treatment could improve the survival and differentiation of CD34+ epithelial stem cells in the bone marrow, as well as their ability to enhance fracture healing. Therefore, the goal of the present study was to evaluate the efficacy of intravenous FAS administration on fracture healing in a small animal model.

METHODS: Unilateral mid diaphyseal femoral fractures were created in 56 male rats (15-month old male Fischer 344) and fixed with an intramedullary nail, approved by the Institutional Animal Care and Use Committee. Rats were treated either with a saline control (2 ml/kg) or Fibrinogen-coated albumin spheres (2 ml/kg = 16 mg spheres/kg), both IV-administered on days 0, 3, 6 and 9 post-fracture. Healing was assessed on days 21 and 35 post-fracture. The newly formed mineralized callus (> 500 mg/cm^3) properties (volume and mineral content) of 3.6 mm long bone segment at the fracture line were quantified by qCT at days 21 and 35 (n=10/sub group). At day 35, the biomechanical properties of the fracture sites, including maximum torque and torsional stiffness were evaluated. Paraffin-embedded, decalcified femur specimens containing the fracture (n=4/subgroup) site were sectioned and stained with CD34 rabbit polyclonal antibodies, peroxidase-conjugated mouse anti-rabbit IgG, DAB substrate, and hematoxylin counterstain. The percentage of CD34+ cells in the mononuclear cell population was counted at 100X magnification, six fields per section. Data were analyzed for treatment efficacy with one-way ANOVA and paired t-test.

RESULTS: Overall, the study group demonstrated faster callus development than control group at the fracture site. At 21 days, the FAS study group was approximately 30% higher in mineral content and mineralized callus volume (p= 0.01) at the fracture site than the control group. (**Table 1**). However, by 35 days, the callus development was comparable for both groups. Specifically, there were no differences in mineral content or mineralized callus volume at the fracture site by day 35. At day 21, neither the control group, nor the study group exhibited measurable stiffness at the fracture site under biomechanical evaluation. However, by day 35, 5 of the 9 control group specimens and 4 of the 9 study group specimens exhibited measurable torsional stiffness and strength under torsional testing. The mean values for maximum torque was higher in study group than control group (164.3 vs 209.7 Nmm, p=0.048); however torsional stiffness was higher in control group than study group (5.89 vs 3.23 Nmm/deg., p=0.023). There was a significant increase in the percentage of CD34+ cells among total mononuclear cells in the 21 day study group compared to the control (4.13 ± 0.28 vs.1.51± 0.15, p<0.0001), as well as in the 35 day groups (4.45 ± 0.43 vs. 2.19 ± 0.2, p<0.0001), (**Figure 1)**.

DISCUSSION: Our results confirmed the previous findings that intravenous FAS administration induces CD34+ stem cell activation and migration via circulation to the injury site during the entire fracture healing period. Further, FAS administration boosted mineralized callus development at the fracture site during the early fracture healing period. However, early administration of FAS did not appear to significantly increase mineralized bridging at the callus site or torsional strength or stiffness at 35 days. This finding was likely affected by the timing and dosage of the treatment drug. While the preliminary data is promising, future studies are needed to evaluate the efficacy of FAS on fracture healing.

SIGNIFICANCE / CLININCAL RELEVANCE: Our results confirmed the previous findings that intravenous FAS administration induces CD34+ stem cell activation and migration via circulation to the injury site during entire fracture healing period and early callus development.[1]

Table 1. Newly formed hard callus at the osteotomy site (3.6 mm long segment) at Day 21 and 35 (mean ± S.D).

Group	Newly Formed Hard Callus only (> 500 mg/cm^3)			
	Callus Volume (mm^3)		Mineral Content (mg)	
	Day 21	Day 35	Day 21	Day 35
Control	11.69 ± 2.01	16.37 ± 2.62	7.50 ± 1.38	10.70 ± 1.91
FAS Group	15.50 ± 4.10	16.29 ± 6.30	9.79 ± 2.57	10.62 ± 4.10
P value	0.011	0.486	0.015	0.479

n=10 for each subgroup (except 21day control group, n=9)

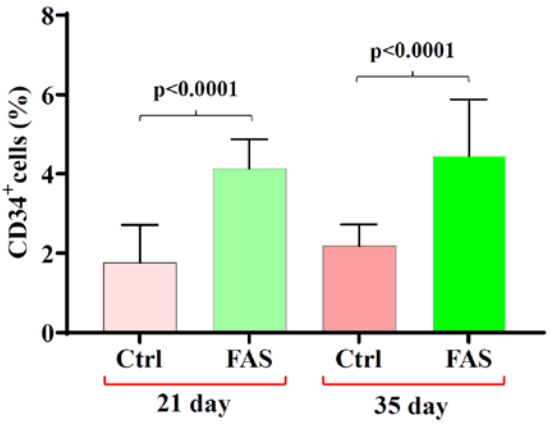

Figure 1. Comparison of CD34$^+$cell ratio (%) in total mononuclear cell population. (n=4/group)

Reference: 1.Mao et al. Radioprotective efficacy of fibrinogen-coated albumin spheres (FAS) against radiation-induced skin damage. 65th Radiation Research Society Annual Meeting, 2019