

SABER™ FORMULATION FOR INTRA-ARTICULAR DELIVERY OF rhGH

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Abstract

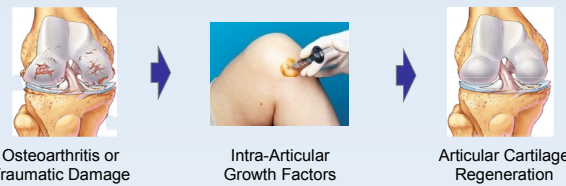
Purpose: To assess the capability of SABER™ formulations of recombinant human growth hormone (rhGH) to extend joint exposure following intra-articular injection for treatment of osteoarthritis.

Methods: SABER formulations of rhGH were prepared as suspensions of spray-dried protein in vehicles composed of sucrose acetate isobutyrate (SAIB), solvents and polymeric excipients approved for parenteral use. These formulations were evaluated *in vitro* for release and stability. Subsequently, the most promising formulations were evaluated for pharmacokinetics (PK) and biocompatibility in beagle dogs. The production of IGF-1 is established in the literature to be a downstream event following stimulation by hGH. Thus, it was used as a biomarker to gauge the effect of the hGH. Following intra-articular injection in the right knee of the hind leg, synovial fluid and serum samples were collected at various time points to determine rhGH and IGF-1 concentrations.

Results: rhGH was chemically and physically compatible with a range of common SABER excipients. Among the formulations studied, two demonstrated particularly promising release and stability profiles *in vitro*. Intra-articular (IA) injection of these formulations (150 µL), at a concentration of 50 mg/mL hGH, provided sustained delivery to the knee post IA injection. The rhGH levels were sustained above a proposed efficacy threshold for two weeks, extending the duration of delivery by about 10-fold compared to unformulated hGH. These formulations maintained a 3-4 log higher concentration in the joint relative to systemic exposure. Biomarker analysis indicated elevated synovial levels of IGF-1 for 2 weeks, confirming the bioactivity of the released hGH. The SABER formulations are biodegradable, and the assessment of biocompatibility is in progress.

Conclusions: SABER depot formulations are promising candidates for continuous local delivery of rhGH to the intra-articular space of the knee.

Intra-Articular Growth Factors & Cartilage Repair



- Growth Hormone Actions
 - Stimulates recruitment, proliferation and differentiation of progenitor cells
 - Stimulates IGF-1 production in differentiating and mature articular chondrocytes
 - Stimulates chondrocyte proliferation and matrix synthesis
 - Morpho-angiogenesis?
- Cartilage Repair – Animal Models and Clinic
 - Sustained focal delivery of hGH to a cartilage defect induced repair
 - Repair of partial-thickness defects in articular cartilage: cell recruitment from the synovial membrane (Hunziker et al. 1996. J. Bone Joint Surg. 78: 721-33)
 - Bolus dose of hGH to rabbit chondral defect enhanced repair
 - Morpho-angiogenesis: A Unique Action of Growth Hormone (AR Dunn 2002. Microvasc. Res. 63:295-303)
 - EquiGen™ delivered SC for equine joint conditions
 - Today some orthopedists use hGH off-label as an OA intra-articular therapy

IA Delivery of rhGH: Efficacy and Safety

- The goal of IA delivery of rhGH via SABER depot was to achieve prolonged therapeutic protein levels in synovial fluid without provoking adverse systemic effects
- Based on receptor affinity and serum levels required for systemic efficacy, we hypothesized that the lower limit of effective concentrations in synovial fluid is 10ng/mL
- To avoid undesirable systemic effects, serum levels should not exceed 1ng/mL, a level that stimulates hepatic IGF-1 production

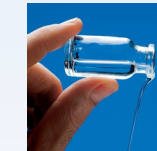
Study Design

Group	Treatment	Route	Dose (mg)	N	Parameters
1	Naked hGH	IV	1.5	4	Systemic clearance
2	Vehicle 1	IA	0	4	Biomarker/PD control
3	SABER-hGH +Zn	IA	7.5	4	IA extended PK/PD
4	SABER-hGH -Zn	IA	7.5	4	IA extended PK/PD
5	SABER-hGH +Zn	SC*	7.5	4	SC extended PK (non-mechanical force)
6	SABER-hGH -Zn	SC	7.5	4	SC extended PK (non-mechanical force)
7	Naked hGH	IA	1.5	4	Baseline PK/PD

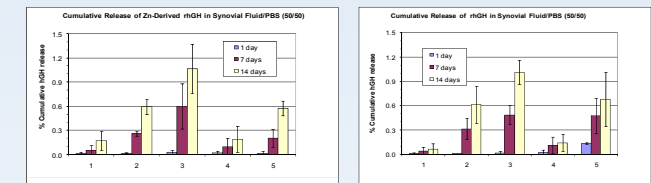
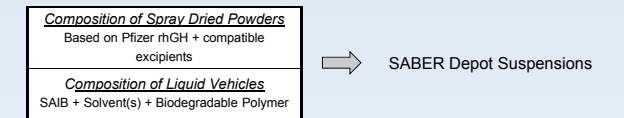
SABER Depot Injectables

Sucrose Acetate IsoButyrate Extended Release

- Parenteral depots for small molecules, peptides and proteins
- Delivery over days to months
- Several mechanisms to control release
- Ability to modulate initial release
- Injectable through fine needles
- High drug payload / low injection volume
- Biodegradable and biocompatible
- Major formulation components / factors
 - Viscous liquid (Sucrose acetate isobutyrate)
 - Hydrophilic and hydrophobic diluents
 - Biodegradable polymers
 - Physical state: solution / suspension / emulsion
 - Physical form of the API

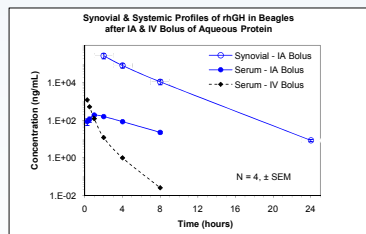


SABER Depot Formulations – In Vitro Release



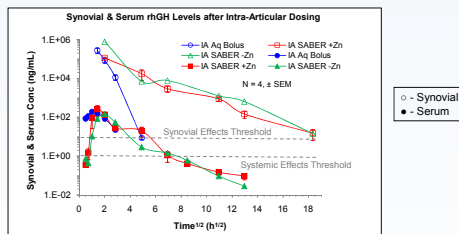
(1) SABER formulation A (2) SABER formulation B (3) SABER formulation C (4) SABER formulation D (5) SABER formulation E

IA & IV Bolus of Aqueous Protein



- Mean rhGH concentrations in synovial fluid and serum after IA injection and in serum after IV injection of 1.5 mg protein/animal as aqueous bolus
- Synovial fluid levels exceeded those in serum and declined more slowly, suggesting that the IA space behaved as a separate PK compartment
- Serum levels after the aqueous IA bolus required 2 hours to reach their peak and were 2-3 Logs lower than levels in synovial fluid. Terminal elimination rates of serum and synovial levels were comparable. Thus, the joint acted as an extended release depot for systemic delivery

IA Delivery of rhGH from SABER Depots



- Synovial fluid concentrations of rhGH derived from IA injection of an aqueous bolus (1.5 mg protein) or SABER depots (7.5 mg protein) are plotted above
- Within 12 hours of injection, the two SABER formulations produced similar synovial profiles that declined ~ 5 Logs over two weeks. Both SABER depots provided extended delivery of rhGH to the joint. The Zn-containing formulation provided superior control of initial release
- A consistent 3 - 4 Logs difference between synovial fluid and serum rhGH concentrations was observed, suggesting the possibility of localizing treatment within a joint, minimizing systemic effects
- The 500-fold difference between synovial and serum $V_{d,ss}$ implies that 5 to 50% of the IA dose escaped to the systemic circulation. Deconvolving the serum profiles indicates the extent of transfer was 30% for SABER depots and 80% for IA aqueous bolus

Non-Compartmental Pharmacokinetic Analysis

rhGH, 1.5 mg/dog as IV Aqueous Bolus								
Dog	C_0 (ng/mL)	AUC (ng*hr/mL)	CL (mL/h)	V_d (mL)	AUMC	MRT (h)	$V_{d,ss}$ (mL)	k_{el} (1/h)
mean	2903.2	978.4	1576.1	1513.5	313.5	0.32	509.5	1.15
sem	360.2	86.7	159.2	337.9	25.5	0.01	63.6	0.18

rhGH, 1.5 mg/dog as IA Aqueous Bolus								
Dog	C_0 (ng/mL)	AUC (ng*hr/mL)	CL (mL/h)	V_d (mL)	AUMC	MRT (h)	$V_{d,ss}$ (mL)	k_{el} (1/h)
mean	1.26E+06	2.36E+06	0.69	1.58	3.54E+06	1.48	1.01	0.45
sem	2.13E+05	4.49E+05	0.15	0.40	8.93E+05	0.14	0.23	0.02

- Values of synovial clearance, CL, and volume of distribution, V_d , are much smaller than for their systemic counterparts. Values for synovial $t_{1/2}$ and MRT are greater

rhGH, 7.5 mg/dog IA SABER depot + Zinc						
Mean	T_{max} (h)	C_{max} (ng/mL)	AUC (ng*hr/mL)	CL (mL/h)	AUMC	MRT (h)
SEM	4	1.13E+05	1.56E+06	0.69	3.14E+07	16.0
	0	1.11E+04	3.20E+05	0.11	5.47E+06	1.0

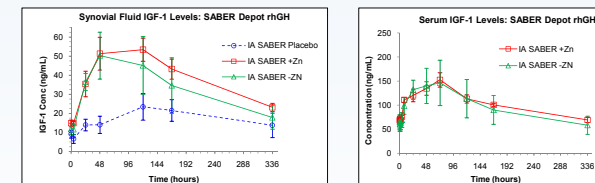
rhGH, 7.5 mg/dog IA SABER depot w/o Zinc						
mean	T_{max} (h)	C_{max} (ng/mL)	AUC (ng*hr/mL)	CL (mL/h)	AUMC	MRT (h)
sem	4	6.56E+05	8.40E+06	0.67	6.24E+07	7.43
	0	2.21E+05	2.93E+06	0.28	3.44E+07	1.39

- NCPK analysis of data from dogs dosed IA with SABER depot formulations (\pm Zn)
- $AUC_{0-\infty}$ declined 4-fold when zinc was incorporated in the formulation, but MRT doubled from 7 to 16 hours, 5-10 fold longer than for IA dosing of aqueous protein

Injection Site	Formulation	BA (%)	SEM (%)	Input Time (h)	SEM (h)
Intra-articular	+ Zn	16.6	4.2	14.5	1.7
	w/o Zn	71.1	28.2	6.0	1.2

- Mean input time ($MRT_{IA, SABER} - MRT_{IA, Aq Bolus}$) also doubled on inclusion of zinc, but was still much shorter than input times for these SABER depots injected SC

Pharmacodynamics: Synthesis of IGF-1



- Increased IGF-1 production is a biomarker for the PD effects of rhGH. Following IA dosing of SABER depot rhGH, synovial fluid and serum levels of IGF-1 were stimulated 2-3 fold over baseline levels for 2 weeks
- Serum concentrations of IGF-1 exceeded levels in synovial fluid at all times and appear to have been stimulated above baseline levels also for about two weeks
- Increased levels of IGF-1 in the synovial fluid may not be due solely to stimulated synthesis in the joint. Transfer from the serum is conceivable, although IGF-1 is protein-bound and cannot diffuse freely between the two compartments

Conclusions & Next Steps

- We have explored SABER depot formulations of rhGH and studied their delivery characteristics following intra-articular (IA) injection into the canine hind knee
- Synovial rhGH levels following an IA aqueous bolus fell 4 - 5 Logs in 24 hours. Following IA injection of SABER depots, the same decline occurred over 2 weeks. Thus, the SABER depots greatly extended delivery to the joint
- A consistent difference of 3 - 4 Logs was observed between synovial fluid and serum rhGH concentrations after dosing with either an aqueous bolus or the SABER depots. This indicates the possibility of local therapy, while minimizing systemic effects
- Following IA dosing of SABER depot rhGH, synovial fluid and serum levels of the biomarker IGF-1 were stimulated 2-3 fold over baseline levels for a period of 2 weeks
- To achieve local efficacy and avoid undesirable systemic effects, the initial release of rhGH from SABER depots should be reduced, without compromising the bioavailability achieved in the present study



Procedures involving the care and use of animals in this study were reviewed and approved by the Institutional Animal Care and Use Committee at LAB Research Inc., in Quebec, CAN. The care and use of animals were conducted in accordance with the current guide to the Care and Use of Experimental Animals as published by the Canadian Council on Animal Care, and the Guide for the Care and Use of Laboratory Animals, an NRC publication. Lab Research Inc. is AAALAC accredited.

