A PRECLINICAL SAFETY STUDY OF INTRAVENOUS INJECTION OF BIOPRESERVATION SOLUTIONS AS A VEHICLE FOR CELLULAR PRODUCTS

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Introduction

Widespread application of cell therapy will require the utilization of preserved therapeutics, as application of fresh products is time and resource intensive. Increased regulatory oversight is anticipated; minimal manipulation techniques and quality of excipient materials are two potential areas of concern. The ability to utilize optimized preservation solutions that are safe to infuse without a wash step would address these concerns.

Although BioLife Solutions HypoThermosol® HTS-FRS and CryoStor™ are cited in numerous IND's and have been utilized in a clinical setting, access to preclinical data supporting these studies is often proprietary and difficult to obtain second hand, as the studies are costly and their outcomes provide a competitive advantage to the customer. Furthermore, the results of such studies are too important not to undertake first hand, whereby the procedure can be well controlled and the outcomes reviewed without bias.

The goal of this study was to collect and present as much quantitative data as possible such that a range of current and future questions related to the safety of systemic delivery could be posed and addressed.

Methods

This study was divided into three stages to evaluate the safety of intravenous injection of HypoThermosol® HTS-FRS (HTS) and CryoStor™ (containing 0, 5, or 10% DMSO as CSB, CS5, and CS10, respectively) into 6-8 week old male C57Bl/6 mice in groups of 5 (Table 1).

Stage I was intended to assess the effects of volume and solution composition; 250µL and 500µL of HTS, CryoStor or controls were injected. Controls consisted of PBS or FHCRC standard freezing medium.

Stage II assessed the safety of injecting 20 million fresh human umbilical cord blood (UCB) cells suspended in the test solutions.

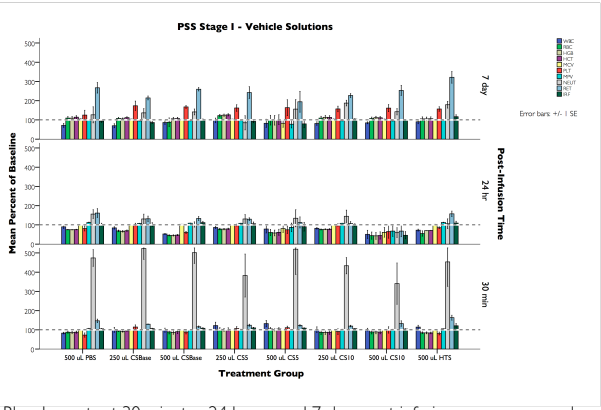
In stage III, UCB cells were cryopreserved in the test solutions, thawed, and injected with no wash.

To assess potential toxicities, complete blood counts were performed on samples collected 1 day prior to injection (baseline), and 30 minutes, 24 hours, and 7 days after injection during necropsy. Organ specimens were collected and processed for histopathology.

	Solution	Injection Volume (µL)	Cells	N
Stage I	CSB	250	N/A	5
	CS5			5
	CS10			5
	PBS	500		5
	CSB			5
	CS5			5
	CS10			5
	HTS-FRS			5
Stage II	PBS	500	20×10^6 (Fresh)	5
	FHCRC			5
	CSB			5
	CS5			5
	CS10			5
	HTS			5
Stage III	FHCRC	500	20×10^6 (Freeze-Thawed)	5
	CSB			5
	CS5			5
	CS10			5
	HTS			5

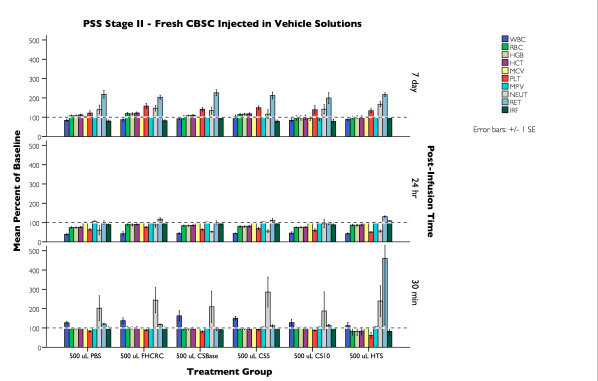
Table 1: Overview of experimental groups by stage.

Stage I Results – Vehicle Solutions



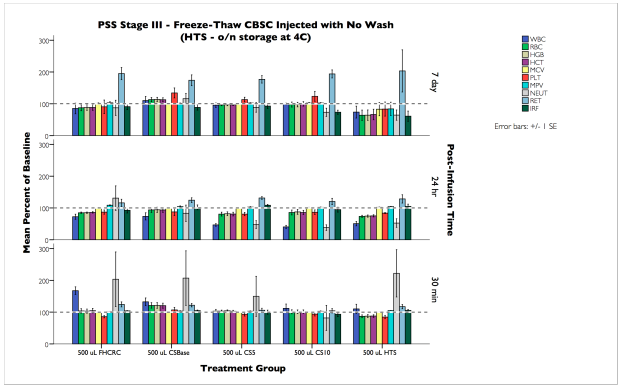
Blood counts at 30 minutes, 24 hours, and 7 days post-infusion were expressed as a percent of baseline (indicated by the dashed line) so that all data could be presented on the same scale. Injection of solutions alone resulted in blood counts that were similar among groups within each time point. In all groups, an immediate spike in neturophil counts was observed at 30 minutes following injection; however, these normalized by 24 hours. Platelets, neutrophils, and reticulocytes were slightly elevated after 7 days. One mortality was observed in the 500µl CS5 group at the time of infusion and was deemed unrelated to the solution composition.

Stage II Results – Fresh Cells in Solution



The addition of fresh human cord blood stem cells produced cell counts similar to vehicle solutions alone. There were no mortalities and all animals gained weight during 7 day follow-up. Again, in all groups an immediate spike in neturophil counts was observed at 30 minutes following injection that normalized by 24 hours. Platelets, neutrophils, and reticulocytes were slightly elevated after 7 days.

Stage III Results – Cryopreserved Cells



Stage III evaluated the safety of no wash infusion of frozen and thawed human cord blood stem cells. 20×10^6 cells were frozen in 500µl, or in the case of HypoThermosol (HTS), the same density of cells were stored overnight at 4°C. All solutions were infused cold (4°C). Again, blood counts were similar between groups for each post-infusion time, there were no mortalities, and all animals gained weight during 7 day follow-up. Similarly, in all groups an immediate spike in neturophil counts was observed at 30 minutes following injection that normalized by 24 hours. Only reticulocytes were slightly elevated after 7 days.

Statistical Analysis

A univariate general linear model with least significant difference (LSD) post-hoc analysis was performed by treatment group for each outcome parameter within each time series of each phase to identify differences between treatment groups. A multivariate general linear model with LSD was also applied to additionally examine homogeneous subsets; in this case, we wanted to evaluate whether there were differences in outcome parameters by post-infusion time within each phase. Statistical differences in the data were observed. In particular, there was an induction of neutrophil and reticulocyte counts within 30 minutes that normalized by 24 hours then became elevated over the next 6 days; these data are difficult to interpret as there was no negative clinical manifestation, therefore they were deemed to be of minimal safety risk at this time.

Summary of Results

- Only one observed mortality that was unrelated to solution composition
- All animals gained weight during 7 day follow-up
- All organs appeared normal upon necropsy
- Blood counts were similar between groups within post-infusion time points
- Slightly elevated platelet, neutrophil, and reticulocyte counts at necropsy

These findings support further clinical investigation into the use of pre-formulated, fully defined; serum- and protein- free HypoThermosol and CryoStor solutions for preservation and as a vehicle for direct injection of cellular therapeutics.