Intra-operative Bone Marrow Processing for Bone Regeneration

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ABSTRACT

The study is the result of the multidisciplinary collaborative efforts of the Departments of Pathology, Apheresis, Orthopedic Surgery and the UC Davis Stem Cell Program.

Background: Processing large amounts of bone marrow aspirate in order to obtain a stem cell concentrate to be used for cellular therapy applications may be performed using apheresis technology. However, a detailed protocol has not been published yet. **Objective:** The aim of our study was to develop a clinical protocol using the CobeSpectra apheresis device (CaridianBCT) to process stem cell products or bone marrow aspirate.

Materials and Methods: In vitro experiments: A total of ten in vitro experiments were performed to assess the feasibility of using CobeSpectra for semiselective processing of mononuclear cells (MNC). In the three experiments whole blood was used for apheresis processing and the MNC recovery was determined. In another seven experiments, the whole blood admixed with stem cell products was processed; in this case the MNC and stem cell recovery were determined. In both types of experiments, pre- and post-processing samples were analyzed for sterility by standard culture methods.

Clinical protocol: The apheresis protocol was used to process bone marrow aspirate in one patient with avascular necrosis (AVN) of the femoral head. The concentrated stem cell product was implanted immediately after processing at the necrosis site and the patient was monitored for two years.

Results: In vitro experiments: The MNC recovery was 66-73% in the vitro experiments with whole blood only. Apheresis processing of whole blood admixed with stem cell products led to a stem cell recovery of 56%. All cultures on pre- and post-processing samples were negative. Clinical studies: The patient tolerated the procedure well and the cultures of the processed bone marrow were negative. The stem cell recovery was 93%. The patient had improved clinical and functional status with preserved joint space without evidence of bone collapse by imaging. **Conclusions**: Bone marrow aspirate may be successfully processed by apheresis within the intra-operative timeframe leading to adequate stem cell enrichment.

BACKGROUND

The regenerative potential of adult stem cells holds a great therapeutic promise. Autologous bone marrow aspirate locally implanted to heal AVN has been previously shown to be a safe treatment that may avoid total hip replacement [1-5]. It has also been known that enrichment for the MNC fraction of bone marrow is more efficient in generating bone and cartilage than unconcentrated bone marrow [6]. The reason for processing bone marrow is to reduce the volume of the aspirated marrow to a feasibly implantable cell suspension containing the cells thought to be responsible for bone regeneration. The technology currently available for bone marrow concentration involves simple centrifugation or density gradient centrifugation by apheresis technology. Simple centrifugation results only in volume reduction without a selection of the stem cell compartment from the bone marrow. Our aim was to develop a protocol using the apheresis technology to obtain small volumes of minimally manipulated enriched stem cell products that can be used for clinical applications in cellular therapy protocols.

MATERIALS & METHODS

Human whole blood collected in CPDA-1 anticoagulant from regular blood donors were purchased from Stanford Blood Bank. **Stem cell products** collected by apheresis after G-CSF stimulation that were not used by the intended patient were donated by BloodSource.

In vitro experiments: A total of ten in vitro experiments were performed using the setup illustrated in **Figure 1**. In the first three experiments only whole blood was processed by apheresis. CBC and culture were performed on the pre- and post-processing samples, and the MNC recovery was calculated. In the following seven experiments, the whole blood was admixed with stem cell products and cell recovery was calculated for both MNC and stem cells.

Determination of the CD34+ cell content was performed at the Stanford Clinical Flow Cytometry Laboratory by the standard dual-stage ISHAGE protocol.

PROTOCOL OVERVIEW

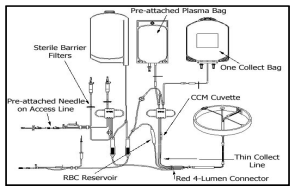

Figure 1. Experimental setup using the CobeSpectra apheresis device.

Bone marrow processing: Aseptic technique is used throughout procedure. Pre and post-processing samples were drawn for CBC, cultures and CD34+ cell content determination. The CobeSpectra Apheresis device was setup with the AutoPBCS Disposable tubing set, primed with 0.9% normal saline and run by using the **AutoPBSC Option**. Standard patient data and current hematocrit of the pre-processing sample performed STAT were entered. The Harvest and Chase volumes were configured as 4 and 6 mL, respectively. The target and run values displayed by the device are overrun to an Inlet value of 2600 mL, Collect value of 27 mL, and Inlet pump flow rate of 65 mL/minute. The access and return lines were connected with an attached veno-trans set to the bag containing the bone marrow aspirate. The run was performed with an Inlet:AC ratio to 50:1. Throughout the processing, the interface was visually monitored and the hematocrit was decreased by 3 % after the first harvest or 1000ml, if necessary, to correct a receding interface. The processing was continued until the target inlet volume was reached. The blood was mixed periodically to prevent clump formation. No rinseback is performed. **Clinical protocol:** One patient with AVN of the femoral head was treated by implantation of enriched stem cell product obtained from the posterior iliac crest aspiration. The marrow processing took place immediately after harvesting with the patient under general anesthesia. The patient was monitored for two years and the clinical data, operative time and overall cost were retrospectively compared to those of patients who underwent a similar procedure except that the aspirate was processed using the Bone Marrow Aspirate Concentration (BMAC) system (Harvest Tech Corp.).

RESULTS

In vitro experiments: The MNC recovery after processing only whole blood was 73% after processing of 3100 mL with three harvest phases versus 66% after processing 2100 mL with two harvest phases (n=3). The **stem cell recovery** determined as (post CD34+cell content x post volume x100)/(pre CD34+cell content x pre volume was 56 ± 22% (n=7). The processing time was 50 minutes on average. **Clinical results** showed functional improvement (absence of pain) with no need for total hip replacement within the two year follow-up. Control patients treated with unconcentrated bone marrow showed signs of disease progression at ten months post-procedure. The MNC and % CD34+ **cell recovery** were 93% when using CobeSpectra. **Processing time and cost:** The use of the described apheresis protocol led to an increased total anesthesia time, but yield per mL of bone marrow harvested was higher and cost per mL of bone marrow processed was lower using the Cobe procedure ($2,000/500 mL versus $3,000/240 mL when BMAC system was used).

CONCLUSIONS

The advantages of apheresis technology are processing of larger volumes of the aspirate and semi-selective concentration of the MNC and stem cell compartment. Bone marrow processing using CobeSpectra apheresis technology can be used to appropriately process bone marrow within the intra-operative timeframe. More studies are needed to define the parameters of the bone marrow product used for implantation and their effect on targeted clinical outcome.

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