Establishing a cord blood banking and transplantation program in Mexico: a single institution experience

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BACKGROUND: Over the past decade, umbilical cord blood (UCB) banking and transplantation have increased significantly worldwide. The experience in developing countries, however, is still limited. In January 2005 the Mexican Institute of Social Security (IMSS) initiated its UCB banking and transplantation program. This study reports on the experience generated at this institution during the first 2 years of activities.

STUDY DESIGN AND METHODS: A public UCB bank was established at La Raza Medical Center, IMSS, in Mexico City. Good manufacturing practices and standard operating procedures were used to address donor selection, as well as UCB collection, processing, and cryopreservation. Based mainly on HLA and total nucleated cell (TNC) content, specific UCB units were thawed, processed, and released for transplantation.

RESULTS: Based on stringent selection criteria, 360 UCB units were collected from January 2005 to December 2006. A total of 201 (56%) units (minimum volume, 50 mL without anticoagulant) were processed and stored. Median values for specific parameters were as follows: volume, 89.9 mL; viability, 94.8%; TNCs, 0.91 × 10^8; CD34+ cells, 3.13 × 10^6; and colony-forming cells, 1.20 × 10^6. During this period, 10 units had been released for transplantation to seven patients (six children and one adult). Engraftment was observed in five patients; four of them were still in remission (114-293 days after transplant). In spite of showing sustained engraftment, one patient died on Day +88. Two patients showed no engraftment and died 29 to 30 days after transplant.

CONCLUSION: The results obtained during this initial period are encouraging and indicate that the UCB banking and transplantation program at IMSS will help to improve already existing hematopoietic cell transplant programs in Mexico. The experience generated at IMSS may be helpful to other institutions, particularly those in developing countries.

More than 30 years ago it was demonstrated that significant numbers of primitive hematopoietic cells are found in umbilical cord blood (UCB). It was not until the late 1980s, however, that UCB was recognized as a rich source of hematopoietic stem and progenitor cells for potential clinical use. In keeping with this notion, the first hematopoietic cell transplant in which UCB was used as the source of hematopoietic cells, instead of marrow, was performed in Paris, France, in 1988. Over the past 15 years significant advances have been made both in the basic biology of UCB-derived stem and progenitor cells and in the clinical application of such cells.

ABBREVIATIONS: CBB = Cord Blood Bank; CFC(s) = colony-forming cell(s); IMSS = Mexican Institute of Social Security; UCB = umbilical cord blood; TNC(s) = total nucleated cell(s).

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UCB transplantation has become a first-line therapy for specific hematologic conditions, particularly in pediatric patients. It is noteworthy, however, that during the past decade its application has expanded to adult patients through the use of large single-unit or double-unit transplants. Also during the past decade, single institutions, as well as multinational organizations and government regulatory agencies, have been actively involved in establishing regulatory issues regarding UCB collection, processing, cryopreservation, and banking. Thus, the use of UCB transplantation for the treatment of hematologic, neoplastic, metabolic, and immune disorders continues to increase. Indeed, it is estimated that approximately 7,000 UCB transplants have been performed worldwide and that more than 200,000 UCB units are currently stored in UCB banks in several countries.

Most of the information reported to date regarding UCB banking and transplantation has come from developed countries (i.e., the United States, several European countries, Canada, Australia, and Japan). In contrast, the experience generated in developing countries is still limited. There is no doubt, however, that UCB banking and transplantation are becoming more relevant in the latter countries due to both ease of procurement and cost. Indeed, large clinical programs involving UCB collection, processing, cryopreservation, and transplantation are already in progress in countries like China and Brazil.

In Mexico, UCB banks have also been established, and UCB transplantation programs have already been initiated in different institutions, both public and private. The Mexican Institute of Social Security (IMSS) is a public, multicentric medical institution distributed throughout the Mexican territory, which covers approximately 50 percent of the nation’s population (i.e., 50 million people). IMSS has created hematopoietic cell transplant programs in several cities and the total number of transplants being performed annually is between 160 and 180. The vast majority of hematopoietic transplants performed at IMSS use mobilized peripheral blood as the source of hematopoietic cells; however, due to both clinical and practical reasons, an increasing interest in UCB transplantation has been generated over the past few years. In January 2005, the IMSS Cord Blood Bank initiated its activities. To date, 360 UCB units have been collected; of those, 201 have been stored and 10 of them have been released for transplantation. Herein, we report the experience generated at IMSS during this initial 2-year period.

**MATERIALS AND METHODS**

The IMSS Cord Blood Bank (IMSS-CBB), located at La Raza Medical Center in Mexico City, initiated activities in January 2005. IMSS-CBB staff consists of seven full-time members and three part-time individuals and includes physicians, laboratory technologists, nurses, and social workers.

**Donor selection**

Donors are full-term babies born to healthy, pregnant women receiving obstetric care at Tlatelolco Obstetrics Hospital and La Raza Obstetrics Hospital, IMSS. Both hospitals are located within a distance of 1 km from the UCB Bank. Informed consent was obtained from every woman after a brief overview on UCB transplantation was presented to her, including that UCB donation was voluntary, nonremunerated, a blood sample would be obtained for infectious disease screening, her medical history would be reviewed, and she would not be notified of the UCB status. All these procedures are based on those reported previously and were approved by the IMSS Medical Research Ethics Committee.

UCB units were considered for collection if: 1) the mother was between 18 and 40 years of age with no pregnancy complications; 2) the mother and her first-degree relatives did not have any hematologic disorder (including neoplasias or marrow failure syndromes), immune disorders, inherited coagulopathy, metabolic diseases, or thalassemia trait; 3) both parents had no history of high-risk sexual behavior and/or drug abuse; 4) there were no body piercing, tattoos, or blood transfusion within 1 year of the delivery; 5) the mother was negative for the presence of hepatitis B or C virus (as determined by a chemiluminescent immunoassay, , Abbott Park, IL), syphilis, human immunodeficiency virus (HIV), and immunoglobulin M against cytomegalovirus; and 6) there was no sign of premature delivery, inborn abnormalities or asphyxia of the newborn.

Directed UCB banking was also considered when a family member had a disorder for which UCB transplant had proven to be beneficial. In case the prospective recipient died, the UCB donations would be released to the public inventory.

**UCB collection and processing**

Collections were performed in utero—in 80 percent of cases—and ex utero, outside the delivery room, in 20 percent of cases. These two procedures were performed as previously described. For both collection modalities, the umbilical cord was cleansed by wiping a large area around the intended insertion site with sterile gauze to clean up excess blood. Next, the cord was scrubbed for 30 seconds with topical antiseptic (i.e., povidone-iodine). The collector inserted the needle—of the attached collection set—into a visible placental vein. Collection usually took between 3 and 5 minutes. Either 150-mL blood bags (Grifols, Barcelona, Spain), containing 25 mL of CPD anticoagulant, or 250-mL blood bags (Baxter Inc., Irvine, CA),
containing 35 mL of CPD anticoagulant, were used as the collection container. Samples of the cord blood product were removed from the tubing for further testing and retention. The UCB unit was then stored on wet ice for transport to the UCB Bank for processing.

UCB units of 50 mL or more (without anticoagulant) were processed within 24 hours of collection. Depletion of red blood cells (RBCs) and plasma, as well as white blood cell concentration, were performed based on the principles described by Rubinstein and coworkers22 and with a top-and-bottom system (Optipress II FDR 4920, Fenwall Division, Baxter Healthcare Corp., Lessines, Belgium). Precryopreservation samples were taken for laboratory studies. Total nucleated cell (TNC) count was conducted in a hematology analyzer (Cell Dyn 3700, Abbott). Cell viability was assessed by use of the trypan blue dye exclusion test. CD34+ cells were enumerated in a flow cytometer (FACSCalibur, Becton Dickinson, San Jose, CA).

Hematopoietic progenitor cells capable of forming colonies in vitro (colony-forming cells [CFGs]) were assayed in methylcellulose-based semisolid cultures (MethoCult, StemCell Technologies Inc., Vancouver, British Columbia, Canada), as previously described.21 Hematopoietic colonies were classified as follows: multipotent (CFU-MIX), colonies containing both erythroid and myeloid cells; and erythroid (CFU-E and BFU-E), clusters and colonies containing more than 20 hemoglobinized cells, grouped in one or several clusters. Myeloid colonies comprised the identifiable subpopulations of pure granulocytic colonies (CFU-G), pure macrophagic colonies (CFU-M), and colonies containing both granulocytes and macrophages (CFU-GM).

After processing, high-quality UCB units were selected according to specific, previously reported criteria,21 and were cryopreserved in an automated liquid nitrogen freezer, designated as the Bioarchive (Thermo- genesis Corp., Rancho Cordova, CA), which computerizes storage and retrieval of UCB units. Cryopreservation was performed by adding a cryoprotectant solution of dimethyl sulfoxide (DMSO) and dextran 40 to the RBC-depleted cord blood unit. The final DMSO and dextran concentrations were 10 and 1 percent, respectively. All banked units were negative for fungal, aerobic and anaerobic microorganisms, and HIV virus. UCB thawing and washing were performed according to previously reported methods.21,22 A polymerase chain reaction–based method (PEL-FREEZ SSP UniTray, Dynal Biotech, Brown, WI) was carried out for high-resolution typing of alleles in HLA-A, -B, and -DR loci and confirmatory typing for donor-recipient pairs before each transplant.

### UCB transplantation

Patient treatment, according to the specific hematologic disease, and pretransplant conditioning regimens were performed according to well-established, institutionally approved protocols. UCB units were selected for transplantation based on TNC content and HLA match between donor and recipient. Neutrophil engraftment was defined as the first of 3 consecutive days at an absolute neutrophil count equal or higher than 500 × 10⁶ per L. Platelet (PLT) engraftment, in contrast, was defined as the first of 7 consecutive days at an absolute PLT count equal or higher than 20 × 10⁶ per L. Data were censored at the time of the last medical consultation, 7 to 10 days before submission of this study.

### RESULTS

#### UCB banking

Based on the selection criteria described in the previous section, a total of 360 UCB units were collected from January 2005 to December 2006. A total of 156 units (43%) were discarded; among them, 136 were discarded before processing due to the following reasons: low volume (<50 mL), 98 units; birth-related newborn problems, 24 units; and clotted samples, 14 units. Twenty units were discarded due to viability of less than 90 percent (12 units) or damage during processing (8 units). The rest (204 units; 57% of the collected units) showed no problems before or during processing and were banked. Health counseling 6 months after birth indicated that one baby presented with congenital heart disease, one had lissencephaly, and a third one had Prader-Willi syndrome; these 3 units were also discarded. Thus, the actual public inventory consisted of 201 units.

As shown in Table 1, the median volume obtained per UCB unit was almost 90 mL; with viability around

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<th>TABLE 1. Characteristics of the UCB units processed and stored at the IMSS-CBB from January 2005 to December 2006*</th>
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* A total of 360 UCB units were collected during the period of study; 159 (44%) units were discarded because they did not meet the inclusion criteria. Results shown correspond to relative or absolute numbers per 201 UCB units considered.
95 percent and a median TNC number of $0.91 \times 10^9$ cells. The median frequency of CD34+ cells was 0.35 percent of the TNCs in each UCB unit, whereas CFCs corresponded to 0.13 percent. It is noteworthy that for every single variable analyzed, significant variability was observed between units. Among the total number of CFCs detected in each UCB unit, almost 60 percent corresponded to erythroid progenitors, whereas myeloid CFCs corresponded to 36 percent and multipotent progenitors corresponded to 6 percent (Fig. 1).

As mentioned previously, during this initial period, and to standardize our working methods and conditions, we included units with a minimum volume of 50 mL (without anticoagulant). The maximum volume collected was 149 mL (Table 1). Thus, we decided to evaluate the content of TNCs, CD34+ cells, and CFCs as a function of the volume collected. Among the 201 units processed and stored, 8 of them had a volume between 50 and 60 mL; 42 had 70 to 80 mL; 35 had 80 to 90 mL; 30 had 90 to 100 mL; and 60 had a volume of greater than 100 mL. Although great variability was seen, particularly for CD34+ cells, we observed a direct correlation between the volume of UCB collected per unit and the absolute numbers of nucleated cells (Fig. 2A), CD34+ cells (Fig. 2B), and CFC (Fig. 2C).

Quality control of UCB units released for transplantation

To date, 10 UCB units have been released for transplantation. One of them (released in May 2005) was banked as a directed UCB unit; the rest (released between April 2006 and December 2006) were part of the public inventory. From each one of the units, a pilot sample was processed to evaluate viability and TNC and CFC content after thaw. Unfortunately, we were unable to assess the content of CD34+ cells, due to the reduced cell number in the pilot samples. As shown in Table 2, a 20 percent loss in TNCs was observed after samples were thawed. In contrast, viability of the recovered cells was close to 100 percent, which was similar to the viability observed before cryopreservation. CFC growth was also similar to the one seen before the freezing procedure. It is noteworthy, however, that in one of the 10 units no colony growth was observed.

UCB transplantation

To date, seven patients have been transplanted at four different IMSS hospitals (three of them located in Mexico City and one in Puebla City, Puebla). Five of the patients were diagnosed as having acute lymphoid leukemia, one had acute myeloid leukemia, and one had Fanconi anemia. All but one were pediatric patients. Table 3 shows a summary of the transplants performed and the outcomes observed. The median number of nucleated cells infused per patient was $3.05 \times 10^7$ per kg (range, $1.21 \times 10^7$-6.71 $\times 10^7$/kg), and HLA match was four to five of six. Regarding this latter issue, it is noteworthy that at the moment of selecting the UCB units for transplantation, we placed major focus on compatibility at the level of the DR and A loci; indeed, in five cases, patient and donor were identical for the DRB1 locus; also in five cases patient...
and donor were identical for the A locus. In contrast, in only one case, patient and donor were identical for the B locus, and in two cases, complete disparity between patient and donor was seen for such a locus. In the rest of cases, loci compatibility was observed for only one allele.

Two of the seven patients (Patients 1 and 4 in Table 3) showed no sign of engraftment and died on Days 30 and 29 posttransplant, respectively. The first one was a directed transplant, whereas the second (an adult patient) was an unrelated transplant in which 2 UCB units were used. It is noteworthy that in both cases, the nucleated cell number infused was less than $2.2 \times 10^7$ per kg. The other five patients showed engraftment and four of them were still alive and in good condition at the time this report was submitted (114-293 days after transplant).

Myeloid engraftment was observed between 7 and 54 days posttransplant, whereas PLT engraftment was detected on Days 12 to 87 posttransplant. It is noteworthy that in all five cases, the nucleated cell count infused was greater than $2.4 \times 10^7$ per kg. Interestingly, in one patient that received nonmyeloablative conditioning (Patient 6 in Table 3), engraftment was not observed after 4 weeks of receiving a single-unit transplant. During such a time, steady, albeit low, numbers of neutrophils (100-300 per $\mu$L) were observed; however, a sudden decrease in absolute neutrophil count was detected by Day 30, suggesting that the hematopoietic activity (endogenous and/or from donor origin) was lost. A second transplant was performed 12 days later, which was a double-unit transplant; this resulted in myeloid and PLT engraftment, observed on Days 54 and 87 posttransplant, respectively, and the patient is currently in good condition. Among these five patients, only one showed Grade I acute graft-versus-host disease (GVHD; Patient 2 in Table 3); the rest showed no GVHD at all. Unfortunately, one of the patients in which engraftment was observed (Patient 7 in Table 3) died on Day +88 due to pneumonia.
During the past decade UCB banking and transplantation have increased in a significant manner worldwide. This is particularly true for developed countries. In developing countries, however, the experience is still limited. Herein we report the results of the UCB banking and transplantation program at the IMSS (the largest public medical institution in Mexico, covering approx. 50 million people), which was initiated in January 2005.

Donor selection criteria used at our institution were based on those reported previously by different international organizations\(^{10,21}\) and were also very similar to those used by the Guangzhou group.\(^{15}\) During this initial 2-year period, 360 UCB units were collected. Of those, 204 (57\%) met the inclusion criteria and were stored. Such a proportion was similar or slightly higher than those reported by different institutions,\(^{10,21,24}\) which indicates that our acceptance rate is within the standard range. A total of 204 UCB units in a 2-year period is certainly a low number when compared to those reported by other groups—for example, the Guangzhou group has banked 4476 units in a 7-year period (639 units per year, in average),\(^{15}\) while the Barcelona Cord Blood Bank has stored 7011 units during 11 years (637 units per year, on average; Netcord Newsletter, January 2007)—however, it is important to consider that this is the initial phase of our program; we have evidence that this number will increase significantly in the following years, as our donor recruitment and UCB collection program improves. Three additional units were discarded 6 months after being banked due to health problems and abnormalities of the donors not evident at birth. Thus, the actual public inventory consisted of 201 units (as of December 31, 2006).

During this initial period, UCB units containing a minimum of 50 mL (without anticoagulant) were considered for processing. Under the collection conditions and standards described here, the volume obtained, as well as the values of TNC, viability, CD34+ cells, and CFCs were within the range reported previously by different groups from the United States of America.\(^{2}\) This suggests that our technical procedures were adequate. As expected, we found a direct correlation between the volume collected and TNC, CD34+ cell, and CFC numbers; it is noteworthy, however, that a great variability was observed, which is also in keeping with previous reports.\(^{13,24,25}\) Such variability was particularly evident for CD34+ cells and CFCs.

Based on the experience generated in some institutions, it has been recommended that UCB units must have a minimum of \(0.8 \times 10^8\) nucleated cells to be stored.\(^{21}\) In our experience, only 49 percent of the UCB units banked achieved such a cell number. According to such a recommendation, and considering the results of the present study, from now on only UCB units with a minimum volume of 70 mL will be processed at the IMSS-CBB.\(^{26}\) We anticipate that most of these units will meet the required cell number (\(0.8 \times 10^7\) nucleated cells) and will be stored for their use in single-unit transplants or in double-unit transplants, which are being performed more frequently both for adult patients and for large children.\(^{27,28}\)

In terms of the progenitor cells detected by colony assays, we observed that the majority of them corresponded to erythroid progenitors, followed by myeloid and multipotent cells. These proportions were similar to those reported previously by our own group and others,\(^{23,25}\) and confirm that, in contrast to marrow, where myeloid progenitors are most abundant, erythroid progenitors comprise the majority of CFC in UCB.

Among the 201 units banked, 10 of them have been released for transplantation. This indicates a consumption rate of 5 percent, which compares favorably to those reported by other centers\(^{9,15}\) (Netcord Newsletter, January 2007). Quality control (QC) tests were performed in samples from all 10 units right after they were thawed. We
observed a 15 to 25 percent loss in TNCs; in contrast, viability and CFC levels were not affected. These results are in agreement with those reported by Laroche and colleagues, who observed that after thaw, TNC numbers were reduced by 20 percent, whereas no significant changes occurred in cell viability or in the content of CD34+ cells and CFCs.

During this initial period (January 2005-December 2006), seven patients received transplants, receiving UCB units from the IMSS-CBB. Two of them showed no engraftment and died 29 and 30 days after transplant. In contrast, engraftment was observed in five patients; four of them are still alive and in complete remission 114 to 293 days posttransplant. Although one patient showed a sustained engraftment, he died on Day +88 due to pneumonia. It is noteworthy that in those patients that showed no engraftment the nucleated cell dose infused was 1.21 × 10^7 to 2.15 × 10^7 cells per kg of body weight (even though one of them received a double-unit transplant), whereas the patients that showed engraftment received a median of 3.38 (2.41-6.71) × 10^7 cells per kg of body weight. According to the standards suggested by some authors and at different international forums, 2.0 × 10^7 nucleated cells per kg of body weight should be the minimum cell dose for a UCB transplant. Some researchers further suggest that such a minimum cell dose should be raised to 2.5 × 10^7 cells per kg of body weight. Thus, confirming these notions, in the present study we observed a correlation between cell dose and engraftment.

It has been previously demonstrated that myeloid and PLT engraftment in UCB transplants are delayed, compared to those observed in bone marrow or mobilized peripheral blood transplants, and this seems to be due to a reduced number of stem and progenitor cells in UCB units. In our experience, myeloid engraftment occurred 7 to 54 days posttransplant, whereas PLT engraftment was detected on Days 12 to 87 posttransplant; these results are comparable to those reported by other groups.

In trying to increase cell dose, especially for adult patients and large children, double-unit transplants are being performed more frequently. In those studies, it has been consistently observed that only 1 of the 2 units administered is sustained long-term, whereas the other is somehow lost. Interestingly, the “winning” unit could not be predicted from its infused cell dose, the degree of HLA disparity with the recipient, ABO group compatibility, sex match, or the order in which each one of the units was infused. In the present study, two of the patients received double-unit transplants. One patient died 29 days after transplant without showing any engraftment, whereas the other is still alive (114 days posttransplant) and in clinical remission. At the moment, however, we have not assessed chimerism in this latter patient, so we do not know whether both units are still present in the patient’s marrow or only 1 unit has engrafted.

Another approach that has been suggested to increase cell dose in UCB transplants is the infusion of ex vivo expanded stem and progenitor cells. The studies reported to date have shown that although infusion of expanded cells into patients did not significantly alter myeloid, erythroid, or PLT engraftment, this procedure is feasible and safe, and thus it may be used in particular cases. We have already developed strategies for the ex vivo expansion of UCB-derived progenitor cells and will soon start working on protocols aimed at the clinical-scale expansion of UCB cells under good manufacturing practices.

Finally, it is important to point out that to establish the UCB bank at our institution we had to overcome both economic and cultural issues; it took more than 20 months from the time the project was presented to the IMSS authorities to the time the UCB bank initiated activities. The cost of establishing the bank was covered by the IMSS itself, together with the National Council of Science and Technology (CONACYT, Mexico); these institutions covered 65 and 35 percent of the total cost, respectively. In cultural terms, this initial 2-year period was difficult because of the lack of knowledge among the general population, regarding UCB banking and transplantation.

Thus, obtaining informed consent from the mothers was difficult at the beginning. This is an area that we have worked out, however, by giving talks at different IMSS hospitals and by preparing informative brochures. It is also important to mention that we are currently working on the analysis of the ethnicity and HLA profiles of the units banked to compare them to registries from other countries (e.g., NMDP).

Although the experience presented herein is still limited, the results obtained during this initial period are encouraging and indicate that the UCB banking and transplantation program at IMSS will help to improve already existing hematopoietic cell transplant programs in Mexico. Evidently, long-term assessments of our UCB program will be needed to evaluate its actual impact. The experience generated at IMSS may be helpful to other institutions, particularly those in developing countries.

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