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Using autologous umbilical cord blood and placental cells for hypoxic-ischemic encephalopathy: an exploratory safety and feasibility study

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ABSTRACT

Introduction. Hypoxic-ischemic encephalopathy (HIE) caused by lack of oxygen and perfusion to the brain can lead to acute neurological damage in newborns. Therapeutic hypothermia (TH) is the most effective and safest treatment. However, mortality remains high with numerous long-term sequelae. Cellular therapies, particularly umbilical cord blood (UCB), are being studied as alternative therapies. The aim of this study is to assess the feasibility and safety of combining autologous cord blood cell infusion with moderate hypothermia.

Population and methods. Twelve infants of 36 weeks gestational age or older, diagnosed with moderate or severe HIE and with umbilical cord blood (UCB) collected were included. UCB was volume-reduced, and up to four doses were obtained. These doses were infused within the first 72 postnatal hours. Time to the first infusion and possible adverse reactions to the infusion were evaluated.

Results. Between 2014 and 2019, 12 infants were included in the protocol (TH + UCB), 9 with a diagnosis of moderate HIE and 3 with severe HIE. In all cases, at least one dose of UCB was obtained for infusion. In all cases, the first dose was infused within 24 hours in every case, and no adverse reactions attributable to the infusion were observed.

Conclusions. The collection, processing, and infusion of fresh autologous umbilical cord blood for use in newborns with HIE are feasible and safe under our conditions.

Keywords: *hypoxic-ischemic encephalopathy; cell and tissue transplantation-based treatment; cord blood; feasibility studies; safety.*

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INTRODUCTION

Hypoxic-ischemic encephalopathy (HIE) is a cause of acute neurological damage in newborns. The incidence ranges from 1 to 8 per 1000 live births in developed countries and increases to up to 25 per 1000 live births in developing countries.[1]

HIE can be triggered by various causes before and during labor. The most frequent sentinel events during labor include fetal bradycardia, placental abruption, umbilical cord prolapse, etc. The most critical antepartum sentinel event is gestational age ≥41 weeks.[2]

Currently, therapeutic hypothermia (TH), whether total body or head cooling, is an effective and safe treatment.[3-6] This intervention does not pose a relevant risk to the infant, provided it is applied in tertiary-level units and follows appropriate cooling and rewarming protocols. However, mortality remains high, especially in severe forms of HIE, and approximately about half of surviving infants develop neurological impairment in their early years.[7,8] In addition, newborns with birth depression often do not benefit from a postnatal placental transfusion, as when immediate umbilical cord clamping is often performed due to the need for resuscitative maneuvers. For these reasons, various approaches are still being explored for new treatments, including cellular therapies.

Umbilical cord blood (UCB) is an essential source of stem and progenitor cells with therapeutic potential.[9]

The effect of UCB administration has been evaluated in numerous studies in animal models of EHI, with beneficial effects.[10-12]

In addition, phase I clinical trials have already been conducted, demonstrating the safety and feasibility of intravenous administration of autologous UCB.[13,14]

In this study, the feasibility and safety of infusing autologous umbilical cord blood cells in combination with moderate hypothermia were evaluated following hypoxic damage to the central nervous system.

Feasibility was specifically assessed based on two-time windows. The first one is the inclusion of infants in HT within the first six postnatal hours. The second one is the ability to collect umbilical cord blood, transport it to the Cord Blood Bank (BSCU, by its Spanish acronym) at Garrahan Pediatric Hospital for processing, and then returned to the Ramón Sardá Maternal and Pediatric Hospital for infusion, ideally within 12 postnatal hours of the baby and up to 24 hours.

Regarding safety, the adverse reactions to intravenous infusion were evaluated.

POPULATION AND METHODS

This protocol was approved by the Ethics Committees of the Hospital Materno Infantil Ramón Sardá, Hospital de Pediatría Garrahan (Protocol N.° 748) and by the Instituto Nacional Central Único Coordinador de Ablación e Implante (INCUCAI) (Resolution N.° 273/13). It is a an open-label phase 1 clinical study of open design, with a standard sample size (n = 12) to assess feasibility and safety.

Newborns delivered at the Hospital Materno Infantil Ramón Sardá were initially eligible if they had a gestational age of 36 weeks or more, presented with moderate or severe IHE and UCB collection. Subsequently, the gestational age criterion was modified to 35 weeks.[2] Clinical criteria for hypothermia include a) UCB or first-hour postnatal gas results with pH ≤7.0 or base deficit ≥-16, b) history of an acute perinatal event, c) Apgar score ≤5 at 10 minutes, and d) need for positive pressure ventilation initiated at birth and continued for ≥10 min.

The presence of moderate or severe encephalopathy was defined by the occurrence of seizures or by the presence of signs in 3 out of the 6 categories of the Sarnat and Sarnat classification: level of consciousness, spontaneous activity, tone, posture, primitive reflexes, and autonomic function.

Exclusion criteria included: a) presence of known chromosomal abnormalities, b) presence of significant congenital malformations, c) severe intrauterine growth restriction (less than 1800 g), d) moribund newborns, e) parents who did not provide consent.

Once diagnosed, patients entered the HT protocol using a servo-controlled mattress (Amrraterm HTF®) which maintains the infant's core temperature at 33.5 ºC (± 0.5 ºC) for 72 hours, followed by a gradual return to normothermia.

Cord blood collection and processing

At the beginning of the protocol, the intervening personnel from both hospitals received training.

In particular, the delivery delivery room staff were responsible for identifying potential cases of HIE and performing the UCB collections. Meanwhile, the BSCU staff validated the cell therapy product's processing, fractionation, transport, and storage of the cellular therapy product for this protocol.[15]

UCB was collected by trained personnel. Immediately after birth and before placenta

delivery, the cord was aseptically cleaned with a sterile cleaning solution. An UCB collection bag approved by the National Administration of Drugs, Food, and Medical Technology (ANMAT, by its Spanish acronym) was used to puncture the umbilical vein and collect the blood by gravity. During collection, the blood was mixed with an anticoagulant (CPD).

Once in the BSCU laboratory, Sepax system equipment was used for automatic processing to reduce the volume of plasma and red blood cells from the unit in a closed system, minimizing the risk of contamination. The final product, a nucleated cell concentrate enriched in mononuclear cells (or buffy coat), was fractionated into up to 4 doses (5 x 10^7 cells/kg) according to the infant's weight, packaged in sterile double-bagged syringes within double sterile bags and stored at 4-8 °C until infusion.

All procedures followed the BSCU's standard operating procedures (SOP), authorized by INCUCAI and accredited by the American Association of Blood Banks (AABB).

The number of total nucleated cells (TNCs), cell viability, the percentage of CD34+ cells and the sterility of the preparation were determined for each unit. In addition, samples were taken from the unit and maternal blood for serology and molecular biology analysis for hepatitis A, B, and C, human immunodeficiency virus (HIV-1 and HIV-2), human T-cell lymphotropic viruses (HTLV-1 and HTLV-2), cytomegalovirus (CMV), brucellosis, syphilis, toxoplasmosis, and Chagas disease.

Infusion

Before each autologous UCB infusion, 1 mg/kg intravenous hydrocortisone was administered. Infants received a maximum of four doses distributed according to the following schedules:[13] A) If the first dose is infused before 12 hours postnatal, the second, third, and fourth doses are infused at 24, 48, and 72 hours postnatal, respectively. B) If the first dose is infused between 12 and 24 hours postnatal, second and third doses are infused at 48 and 72 hours, respectively.

If fewer than four doses were obtained, the doses will be infused according to the same schedule and times.

Monitoring and follow-up of patients

Standard monitoring include vital signs, metabolic status, and assessments of respiratory, cardiovascular, renal, neurological, and hematological conditions. In addition, a record of adverse reactions, if any, to the blood infusion is included.

For follow-up assessments, the following were proposed: 2 magnetic resonance imaging (MRI) between 7-28 days post-birth (preferably between 7 and 14 days) and between 4-6 months; visual and audiological analysis; psychometric tests at 4-6 months and 9-12 months; and neurological examinations.

Among the tests performed, we highlight the Infant Learning and Development Test (TADI, by its Spanish acronym),[16] screening test conducted at 3, 6, 9 and 15 months that allows the evaluation of development and learning according to 4 dimensions: language, cognitive, socio-emotional, and motor skills. The results are categorized as superior, normal, at risk, or delayed. Secondly, the Bayley III diagnostic test was administered,[17] between the ages of 12 and 18 months, assessing cognitive, motor, and linguistic development.

RESULTS

Between 2014 and 2019, 12 infants were included in the protocol (TH + UCB), with 9 diagnosed with moderate HIE and 3 with severe HIE (*Table 1*). The 3 infants with the severe form of the pathology died in the intensive care unit. The mean gestational age was 38-39 weeks; 8/12 deliveries were vaginal and 4/12, cesarean sections. Notable sentinel events included gestational age (4/12) and fetal bradycardia (3/12).

Characteristics of the units

In all cases, at least 1 dose of cord blood was obtained. The average collected blood volume was 44.8 ml (with a minimum of 24 ml), and the average TNC count was 50.16 x 10^7 cells (with a minimum of 14.38 x 10^7 cells). In 11/12 cases, viability exceeded 95% (*Table 2*).

Regarding sterility, blood cultures for aerobic and anaerobic microorganisms post-processing were negative (12/12). Additionally serology and molecular biology analysis for transmissible infections showed positive results for syphilis in 2 cases (HI-13 and HI-14, HI: internal patient code). Both mothers tested positive during pregnancy, indicating recent infection, and thus received treatment.

Table 1. Summary of cases included in the protocol

HI	Year	Gestational age	Weight (g)	Delivery	Sentinel event	Encephalopathy	Discharge
02	2014	39	2990	Vaginal	FB	Mild	Alive
03	2015	35	2265	CS	DPPNI	Mild	Alive
04	2016	36	2765	Vaginal	FB	Mild	Alive
08	2016	40	3724	Vaginal	No	Mild	Alive
09	2016	41	3320	CS	PL	Severe	Died
06	2017	40	3400	Forceps	PL	Mild	Alive
11	2017	41	3625	Vaginal	FB	Mild	Alive
12	2018	37	3150	Vaginal	PC	Severe	Died
13	2018	41	3885	Vaginal	No	Mild	Alive
14	2018	40	3060	CS	MSAF	Severe	Died
16	2019	36	2380	CS	DPPNI	Mild	Alive
17	2019	41	4150	Vaginal	PL	Mild	Alive

CS: cesarean section, DPPNI: premature detachment of a normoinserted placenta, FB: fetal bradycardia, HI: internal patient code, MSAF: meconium-stained amniotic fluid, PC: prolapsed cord, PL: prolonged labour.

Table 2. Characteristics of the umbilical cord blood units

	Mean ± SD n (%)
Vol. collected (ml)	44.8 ± 10.47
TNC (x 10^7)	50.16 ± 24.78
Viability (%)	97.22 ± 1.97
CD34 + (%)	0.51 ± 0.29
N° of dosage	
4	3 (25)
3	4 (33.3)
2	4 (33.3)
1	1 (8.3)
Time at the 1st infusion (h:m)	8:16 ± 3:05
<12 h	10 (83)
<24 h	2 (17)

h:m: hour:minutes, SD: standard deviation, TNC: total nucleated cells.

Feasibility

Feasibility depends on the involvement of different actors with a limited time window: 6 hours to initiate TH and up to 24 hours for the infusion of the first dose. As shown in *Table 2* in 10/12 cases, we were able to complete the process within 12 postnatal hours. In 2 cases where the births occurred at night (23:46 and 23:25) on Saturday and Sunday, respectively, we managed to meet the 24-hour time window.

Security and tracking

No acute adverse reactions were observed attributable to autologous blood infusion.

Regarding follow-up, of the 9 babies who were discharged alive, one moved away (HI 04), and two (HI 03 and HI 13), still under follow-up, showed poor adherence to the scheduled check-ups. The goal was to perform at least 2 MRIs, one in the first month of life and another between the ages of 4 and 6 months. All the infants completed this study during the first month (one at 1 month and 6 days), with normal results in 6 cases and pathological in the remaining 3 (*Table 3*). Notable lesions included hyperintensity in the basal ganglia and both thalami, a lesion in the periventricular white matter injury, and injury in the posterior area of the hippocampus with restriction in the diffusion sequence. None of the scheduled MRIs between 4 and 6 months were performed.

Auditory studies showed normal results in 6 out of 8 cases. Only one patient was diagnosed with moderate bilateral mild sensorineural hearing loss, while another showed alterations in the tracing configuration. Visual examinations revealed normal results in 3 children, while

Table 3. Patients' follow-up

HIE	MRI		Auditory examination	Visual examination	Psychometric test		Neurological examinations	Other studies
	7-28 days	4-6 months			4-6 months	9-12 months	Results	Type: result (date)
HI-02	Normal	-	Normal	Normal	Normal	At risk	Normal	EEG: Normal
HI-03*	Pathological	-	Pathological	Pathological	At risk	At risk	Pathological	EEG: Pathological (2015) Normal (2016)
HI-04**	Normal	-	-	-	-	-	-	
HI-08	Pathological	-	Normal	Pathological	At risk	At risk	Normal	EEG: Pathological
HI-06	Normal***	-	Normal	Normal	Normal	Normal	Normal	EEG: Normal
HI-11	Normal	-	Pathological	Pathological	At risk	Normal	Normal	EEG: Normal
HI-13*	Normal	-	Normal	Normal	-	-	Normal	EEG: Pathological
HI-16	Normal	-	Normal	Pathological	At risk	At risk	Normal	EEG: Pathological
HI-17	Pathological	-	Normal	Pathological	Normal	Normal	Normal	EEG: Pathological

** poor adherence; ** they moved out; *** 1 month and 6 days; EEG: electroencephalogram; HIE: hypoxic-ischemic encephalopathy; MRI: magnetic resonance imaging.*

5 showed abnormalities in latency and amplitude values for the P100 wave.

In developmental assessments, psychometric studies between the ages of 4 and 6 months, normal results were observed in 3/7 cases. Between 9 and 12 months, 3 out of 7 also had normal results, with 2 babies maintaining the level (HI-06 and HI-17), 1 improved (HI-11) and 1 deteriorated (HI-02) (*Table 3*). Pathological examination was considered as the appearance of motor disorders or poor head circumference growth progression.

In physical neurological exams, 7 out of 8 cases showed normal results. Analyzing the electroencephalograms, 5 out of 8 had pathological results, with only one reverting to normal in a subsequent EEG (*Table 3*). Four children were diagnosed with focal epilepsy according to ILAE 2017 criteria,[18] with 3 of them experiencing resolution of clinical seizures.

DISCUSSION

Our results show that the collection, processing, and intravenous infusion of fresh autologous UCB reduced in volume and red blood cells is feasible, within the specified time windows and safety under our conditions.

Therapeutic hypothermia is the best available treatment for infants with moderate or severe HIE. However, mortality remains high; and surviving infants present moderate or severe neurological developmental disorders, with no improvement in these rates in recent years in low- and middle-income countries.[8,19]

These data have motivated research into various strategies, particularly cellular therapies. In this regard, numerous preclinical studies have shown improvements with the use of UCB,[12] while a meta-analysis suggested that mesenchymal stem cells (MSCs) could improve neurological function.[20] Based on these results, phase I and II clinical trials have been developed with both UCB[13-14] and MSCs.[19]

Despite efforts, no results are yet available from randomized trials (phase III) still need to be evaluated, evaluating the beneficial effects or harms of these interventions for preventing morbidity and mortality following HIE in newborns.[9]

Beyond the discussion on the cell type, this experience has allowed us to corroborate relevant points when designing and conducting clinical trials.[21] Firstly, standardizing clinical trial variables will enhance the power of the results and, consequently, improve their interpretation. Our protocol is based on the protocol published by Cotten et al.[13] regarding the number of doses (up to 4), infusion times (12, 24, 48, and 72 hours), and defined concentration of nucleated cells. However, it differs from that published by Tsuji et al.,[14] which consists of 3 doses within the first 72 hours, with variable concentrations (total cells obtained divided into 3 doses). We also observed differences in the parameters evaluated both during the intervention and follow-up, wich made the results not comparable.

Second, context assessment is essential for the development of implementation strategies

and their constant review to achieve objectives. Cotton[13] emphasized the importance of effective communication within multidisciplinary teams, while Tsuji[14] emphasized the assessment of the context, particulary where and how risky deliveries occur. In our case, we emphasize both, communicaton among stakeholders and the evaluation of the context and resources, such as the availability of slots for the planned studies.

While further studies are needed to explore whether UCB or any of the cell types have any beneficial effect for patients with HIE, the standardization of clinical trials, effective training and communication among stakeholders, and improvements in implementation strategies will allow us to continue advancing in the search for new therapies.

CONCLUSIONS

Based on the results obtained, we can conclude that collecting, processing, and infusing fresh autologous umbilical cord blood for use in newborns with HIE are feasible and safe under our conditions.

Effective communication among the different actors, continuous training, and assertive assessment of the context and available resources are key factors in defining implementation strategies for protocols.

The standardization of variables in clinical trials of cellular therapies will enhance the interpretation of results, optimizing resource utilization. ■

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