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Original Article

Allogeneic human umbilical cord blood for acute ischemic stroke: Phase I clinical trial

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Abstract

Objectives: Transplantation of human umbilical cord blood cells (hUCB) may enhance neuroprotection, and thus, the intravenous (IV) infusion of hUCB in patients with acute ischemic stroke (AIS) is being tested for its safety and efficacy. **Materials and Methods:** We conducted a 12-month, open-label, and single-center, phase I trial of hUCB treatment in AIS patients at the age of 45–80 years, with magnetic resonance imaging evidence of acute infarction in the internal carotid artery supplied territory and the National Institute of Health Stroke Scale (NIHSS) score between 6 and 18. Eligible participants received a single-dose IV infusion of hUCB followed by the two doses of mannitol infusion within 9 days after the onset of stroke symptoms. The primary endpoint was the incidence of adverse events (AEs) and the secondary endpoints were the changes in NIHSS, Barthel index (BI), and Berg Balance Scale (BBS) scores. **Results:** Six patients (Male: Female = 3: 3) were enrolled with a mean age at 65.8 years. A total of 40 AEs occurred in six participants during this study, which included nine serious adverse events. Only transient erythema multiforme and hematuria were probably and possibly related to hUCB infusion, respectively. The mean NIHSS score was 11.5 at baseline and it significantly improved at 1, 3, 6, 9, and 12 months after treatment (mean change from baseline: −4.0, −5.3, −6.8, −7.0, and −7.3). The mean BI score was 22.5 at baseline and it significantly increased at 3 and 6 months after treatment (mean change from baseline: 26.7 and 42.5, respectively). The BBS score increased numerically but did not reach statistical significance. The changes in cytokine levels and spleen size were unremarkable. **Conclusion:** The IV hUCB was safe and well tolerated in AIS patients, and the preliminary efficacy results demonstrated its therapeutic potential, supporting the conduct of a randomized, placebo controlled, phase II clinical trial in future.

Keywords: *Acute ischemic stroke, Adverse events, Human umbilical cord blood, National Institute of Health Stroke Scale, Phase I clinical trial*

Introduction

Acute ischemic stroke (AIS) is the third-leading cause of death and disability combined globally. The common use of antiplatelets or anticoagulants is mainly for ischemic stroke prevention but of limited value in acute treatment. Although intravenous (IV) thrombolysis with recombinant tissue-type plasminogen activator (rt-PA) and intraarterial device-based therapy for AIS have been well developed, the narrow therapeutic time window within 3–4.5 h, the complication of post-rt-PA intracerebral hemorrhage, and the high technical threshold of endovascular thrombectomy remain the major barriers to treatment. The development of treatment options within the days of AIS is much needed when salvage of ischemic brain cells may still be possible.

The human umbilical cord blood (hUCB) consists of hematopoietic progenitors with higher availability for transplantation and lower risk of graft-versus-host disease (GVHD) than bone marrow transfusion [1]. The hUCB

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monocytes, when IV infused in the rat model of AIS, reduced the levels of proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukins (IL)-1 β, and IL-2, and as a result, enhanced neuroprotection after stroke [2]. Delivery of hUCB has been shown to reduce infarct volume and improve functional outcomes in the animal models of stroke [3].

There have been a few trials of autologous stem cells from bone marrow delivered intravenously for patients with ischemic stroke in which favorable safety profiles were reported. Laskowitz *et al*. conducted a phase I safety study of a single infusion of allogeneic hUCB for adults with ischemic stroke of middle cerebral artery at 3–9 days poststroke, and their results showed no serious adverse effects directly related to hUCB and all participants had functional improvement at 3 months posttreatment [4]. The objective of our study was to enroll six AIS patients to evaluate the efficacy and safety of IV hUCB treatment delivered within 9 days of stroke onset in a phase I clinical trial.

Materials and methods

Study design and overview

This is a single-center, open-label, phase I clinical trial to study the safety and feasibility of allogeneic hUCB into six adult patients with AIS. Eligible subjects received a single-dose IV administration of hUCB followed by two doses of mannitol infusion. The subjects were then followed-up for 12 months. The efficacy analysis was evaluated by the National Institute of Health Stroke Scale (NIHSS), Barthel Index (BI), Berg Balance Scale (BBS), brain magnetic resonance imaging (MRI), and spleen size, while the safety was assessed by evaluating the adverse events (AEs) and serious adverse events (SAEs) as well as the laboratory test results throughout the study. The reason to choose BBS (0–56) over the modified Rankin scale: 0–6 is because it provides more details of the function status, particularly balance or low body function. The first patient was enrolled during 2018–2019, and due to the trial team transfer, the second to sixth patients were enrolled during March to June 2022.

Study participants

Eligible patients were male or female 45–80 years of age who experienced an AIS of anterior circulation (internal carotid artery supplied territory) without midline shift or hemorrhagic transformation and the NIHSS score was between 6 and 18 at the baseline. Pregnant or lactating female, participants with medical conditions of impaired liver function, cancer, acquired immunodeficiency syndrome, or other immunological diseases requiring immunosuppressants, and those who cannot tolerate MRI scans would be excluded. During the screening phase, we further excluded patients with NIHSS score decreased ≥4 points between the baseline and the second evaluation day of visit 1, those who received rt-PA in the first place or under other interventional clinical trials, and human leukocyte antigen (HLA) typing match fewer than 4 out of 6. The use of immunosuppressants and any investigational drugs were prohibited before screening until the end of follow-up period.

Study procedure

This study is of sequential enrollment that the second patient is enrolled on the condition that the first patient does not encounter any safety issue within the 1st month of the single hUCB infusion. All six subjects were enrolled from the neurology service in Hualien Tzu Chi Hospital, Taiwan. During the screening phase, eligible subjects underwent function assessments and various blood tests including HLA typing. The hUCB infusion was scheduled to be completed within 9 days of stroke onset. After informed consents were obtained and all enrollment criteria were met, the provider StemCyte, an accredited UCB banking company with branches in the United States and Taiwan, would initiate the HLA-matching process and select the candidate UCB for infusion. A single-dose infusion of hUCB (75–100 mL with 2–5 × 10^8 cord blood mononuclear cells) was administered intravenously within 45 min and followed by 20% mannitol 200 mL for 30 ± 10 min twice with an 8 ± 2 h interval. The use of mannitol was intended to open blood–brain barriers and allow the potential hUCB cells to flux into ischemic regions as salvage.

All other medications required for stroke control and prevention, in the judgment of the investigator, were not prohibited. Thrombolysis therapy with rt-PA was not allowed for AIS treatment in the trial. This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board of Hualien Tzu Chi Hospital (IRB105-71-A) and registered with www.ClinicalTrial.gov identifier NCT02433509. Written informed consent was obtained from all research participants and his/her proxy informant.

Human umbilical cord blood cell source, selection, and transportation

The hUCB is provided by StemCyte. The setup of StemCyte Cord Blood Bank was approved by the Food and Drug Administration in January 2010. The hUCB collection, processing, storage, and release process of StemCyte have been accredited by the Association for the Advancement of Blood and Biotherapies since 2004. StemCyte has been accredited by The Foundation for the Accreditation of Cellular Therapy in fulfilling the international standards for the collection, processing, testing, storage, screening, and claiming process of the allogeneic and autologous hUCB in June 2012.

The selection of donor recipient is based on the matching of ABO blood groups and HLA typing at least 4 out of 6. The selection of dose in this study was based on an autologous bone marrow nuclear cell study in ischemic stroke subjects [5], in which patients who received 1–5 × 10^8 bone marrow mononuclear cells did not exhibit any complications or AE during the 180-day follow-up, demonstrating its safety and feasibility, and thus, we selected 2–5 × 10^8 mononuclear cells/75 mL in this study. The frozen hUCB was transported to the study site in a liquid nitrogen tank (≤−150°C) once the treatment date was confirmed. The hUCB was thawed in a 37°C water bath by well-trained staffs at the study site before IV administered to subjects.

Endpoints

The primary endpoints are the safety variables the incidence of AEs and SAEs following hUCB infusion and throughout the study as well as any significant change in the hematology and biochemistry laboratory assessments from baseline to different timepoints. The AEs were coded with MedDRA® (version 25.1) and further classified based on the system organ class, preferred terms, severity, and relationship with treatment. The severity of AEs was defined by the Common Terminology Criteria for AEs, whereas SAEs were determined by death, life-threatening conditions, hospitalization, permanent damage, or disability. The secondary endpoints include changes in efficacy evaluated by NIHSS, BI, BBS, and brain MRI at several timepoints from baseline till the end of the study at 12 months after treatment. The NIHSS is composed of 11 items and used to objectively rate the severity of ischemic stroke. The NIHSS score ranges from 0 to 42, with higher scores indicating greater severity of stroke. The BI is used to assess functional dependence in the activities of daily living, covering bathing, climbing stairs, dressing, mobility, transfer, feeding, toilet use, grooming, bladder, and bowel function. The BI yields a score of 0–100, with higher scores indicating greater functional independence. The BBS consists of 14 items to test functional balance, with a score ranging from 0 to 56, and higher scores indicate greater balance. The changes in the cytokine levels IL-2, IL-6, IL-10, IL-17, interferon (IFN)-γ, and TNF-α and spleen size measured by the abdominal sonography are set to be the exploratory endpoints.

Data quality assurance

To ensure the study was well conducted, the following procedures were performed.

Site initial visit

Before the start of the study, the sponsor scheduled a site initial visit to train the investigators and site coordinators on protocol, informed consent process, International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice guidelines, AE and SAE reporting, source documentation and case report form (CRF) filling, maintenance of the investigator site file, clinical supplies dispensing, and accountability and storage procedures.

Monitoring

During the study period, the designated monitors visited the sites to ensure the feasibility of the site, the compliance to the protocol, the accuracy of the data between the medical charts and CRFs, and the maintenance of the study-related files. The CRFs were sent to the center of data management only after being verified by the monitors.

Data handling

Double data entry, data verification, and data validation were performed periodically after receiving CRFs from the sites. The data manager generated queries using data clarification forms to the site personnel when there were any potential errors, omissions, or unlikely values in the data. After confirming all discrepancies and resolving all queries, the database was locked by the data manager in line with the standard operation procedures, and the analyses were conducted using the SAS® software (SAS® Institute Inc., USA, version 9.4).

Statistical analysis

In addition to using descriptive statistics to characterize the demographic and clinical features of our six participants, we employed generalized estimating equations (GEE) to examine the longitudinal effects of hUCB on NIHSS, BI, BBS, and cytokines while adjusting for age, sex, and HLA-matching status.

Results

Six participants were enrolled sequentially with a mean age at 65.8 years and male-to-female ratio of 1:1 [Table 1, the starting subject number was 114-02 because the first subject 114-01 failed during the screening phase when subject 114-02 had been registered]. Participants received IV hUCB infusion at a mean of 6.8 days after stroke onset. Four participants (66.7%) experienced right brain infarction, whereas two subjects experienced left brain infarction. Hypertension (n = 3, 50%) appeared to be the most common past medical condition, followed by hyperlipidemia.

Primary endpoint: Safety

The primary endpoint of this open-label phase I trial was safety based on the occurrence of study-related AEs or SAEs. By the end of 12-month follow-ups, there were a total of 40 AEs, and of which 19 (47.50%) events were mild, 11 (27.5%) events were moderate, 5 (12.5%) events were severe, and 5 (12.5%) events were life-threatening [Supplemental Table 1]. For the causality of AEs, only 1 (2.5%) and 4 (10.0%) AEs were probably and possibly related to hUCB infusion; nevertheless, these five AEs were all mild in severity. The AE probably related to hUCB infusion was erythema multiforme, which had resolved without any action taken; whereas the four possibly related AEs (all were hematuria after mannitol infusion) had also resolved within a day. With respect to hematuria, the IV infusion rate and dosing frequency of mannitol were adjusted according to the patient's conditions. No clinically significant safety trends were noted.

A total of nine SAEs requiring hospitalization were reported during the 12-month course of follow-ups, such as urinary tract infection, renal stone, gastrointestinal tract bleeding, and liver tumor. These events took place long after and were considered not related to hUCB infusion. Benign prostate hypertrophy required longer time to develop symptoms and so unlikely resulted from hUCB [Table 2].

Secondary endpoint: Efficacy

The trends of NIHSS, BI scores, and BBS demonstrated improvement of neurological and motor functions over time. The changes in NIHSS from baseline to 1, 3, 6, 9, and 12 months after treatment were all statistically significant, whereas the improvement of BI scores was only significant at 3 and 6 months after treatment. Nearly all subjects showed improvement in BBS.

Table 1: The umbilical cord blood profile and functional status of stroke patients in the trial

Subject number	Baseline characteristics										
	Time to hUCB infusion after stroke onset (days)	Age	Sex	NIHSS	TOAST	BI	BBS	CD34 (10^6)	Number of HLA matching	MNC (10^7)	TNC (10^8)
114-02	9	46	Male	8	2	0	0	1.1	6/6	2.6	65.3
114-03	5	67	Female	11	3	30	0	3.9	5/6	4	123.2
114-04	7	78	Female	18	1	5	0	8.8	4/6	4.4	134.3
114-05	8	68	Female	10	3	50	3	2.6	5/6	4.1	109.4
114-06	7	65	Male	6	3	40	0	5.2	5/6	4.4	112.4
114-07	5	71	Male	16	1	10	0	1.4	3/6	4.3	126.7
Mean±SD	6.8 (1.6)	65.8 (10.7)		11.5 (4.6)		22.5 (20.4)	0.5 (1.2)	3.8 (2.9)		4.0 (0.7)	111.9 (24.6)

TOAST classifications were categorized in 1: Large-artery atherosclerosis, 2: Cardioembolism, 3: Small-vessel occlusion, 4: Stroke of other determined etiology, and 5: Stroke of undetermined etiology. hUCB: Human umbilical cord blood, HLA: Human leukocyte antigen, MNC: Mononuclear cell, TNC: Total nucleated cell, NIHSS: National Institute of Health Stroke Scale (score: 0–42), TOAST: Trial of org 10,172 in acute stroke treatment, BI: Barthel index (score: 0–100), BBS: Berg Balance Scale (score: 0–56), SD: Standard deviation

Table 2: Serious adverse events after human umbilical cord blood infusion

Subject number	SAE	Time since hUCB infusion (days)
114-07	Urinary tract infection	80
114-06	UGI bleeding	221
114-04	Right renal stone	246
114-07	Pneumonia	308
114-07	Bladder tumor	315
114-06	Liver tumor	326
114-06	Cholangitis	326
114-06	Gastric cancer (adenocarcinoma)	332
114-07	Hyperplasia of prostate	342

SAE: Serious adverse event, hUCB: Human umbilical cord blood, UGI: Upper gastrointestinal

Six patients with AIS had a mean NIHSS score of 11.5 ± 4.6 at baseline. The mean NIHSS score started to improve significantly at 1 month after treatment and continued to improve at 3, 6, 9, and 12 months after treatment (−4.0 ± 1.8, −5.3 ± 1.8, −6.8 ± 1.7, −7.0 ± 1.8, and −7.3 ± 2.1, respectively, $P = 0.031$). All participants had decreased NIHSS scores from baseline [Figure 1].

The mean BI score was 22.5 ± 20.4 at baseline, and it gradually increased from 24 h to 1 month after treatment, and continuously increased significantly until 6 months after treatment (26.7 ± 12.1 at 3 month and 42.5 ± 24.9 at 6 month, $P = 0.031$), but stabilized thereafter until the end of the trial. The mean BBS score was 0.5 ± 1.2 at baseline and gradually improved to 7.7 ± 11.4, 15.5 ± 16.1, 24.2 ± 21.9, 25.7 ± 23.7, and 27.2 ± 24.5 at 1, 3, 6, 9, and 12 months after treatment.

In the GEE models, we found that the improvement of NIHSS, BI, and BBS scores was not only associated with time but also the HLA-matching status [Table 3]. Images of brain MRI were all well taken at the scheduled timeline, except subject 114-06, who did not complete the last visit. The diffusion-weighted images of brain MRI of the six patients revealed acute infarction at baseline, followed by T2-FLAIR images at each time point, showing poststroke structural changes over time [Figure 2].

Exploratory endpoint

Our participants had highly variable cytokine levels of IL-2, IL-6, IL-10, IL-17, IFN-γ, and TNF-α (0.49 ± 0.70 pg/mL, 4.03 ± 9.20 pg/mL, 1.59 ± 1.80 pg/mL, 4.91 ± 3.88 pg/mL, 4.89 ± 3.41 pg/mL, and 45.91 ± 24.10 pg/mL, respectively). Only IFN-γ was found to increase over time in the GEE models, and otherwise, no trend of change was identified [Table 3]. The spleen size measured by the ultrasound appeared to be unchanged.

Discussion

The single-center, open-label, phase I trial of single-dose hUCB IV infusion for patients with AIS within 9 days of stroke symptom onset demonstrated its safety and potential efficacy after 12 months of follow-ups. Most AEs were mild in severity and unrelated to hUCB. Only erythema multiforme and hematuria were considered related to hUCB and both resolved within a day. The NIHSS, BI, and BBS scores all improved over time and the improvement was associated with HLA-matching status. The cytokine profile and spleen size were unremarkable, suggesting that no significant inflammatory responses were induced by the single-dose hUCB.

The primary objective of this study was to evaluate the incidence of AEs and SAEs following hUCB infusion. The most common AE during the day of hUCB infusion was gross hematuria ($n = 4$), which likely resulted from rapid mannitol infusion with blood osmotic surge, leading to the injury of kidney tissue, together with the concurrent use of antiplatelet or anticoagulant for stroke prevention. The use of mannitol in combination with hUCB infusion would help opening the blood–brain barrier, and therefore, much smaller amount of hUCB is required to achieve the same effect of neuroprotection, making the efficiency of cell transplantation greatly improved [6-8]. Hematuria is a common transfusion reaction after people receive an infusion of frozen-thawed hUCB [9]. Although hemorrhagic cystitis is known to be a common complication of allogeneic hematopoietic stem cell transplantation, and up to 68% of patients with hemorrhagic cystitis would develop gross hematuria [10], hematuria in our participants took place during or right after mannitol infusion, which could not be explained by an inflammatory process.

The safety of allogeneic stem cells in AIS patients has been widely demonstrated in previous studies [4,11-15]; however, it remains the

Table 3: Longitudinal changes of functional status and cytokines after adjusting for age, sex, and human leukocyte antigen-matching status in the generalized estimating equations models

Item	Age, β (95% CI)	Sex (male vs. female), β (95% CI)	Number of HLA matching, β (95% CI)	Time, β (95% CI)
Function				
NIHSS	0.02 (−0.25–0.29)	−5.06 (−7.81–−2.30)**	−3.60 (−5.20–−2.01)**	−0.02 (−0.02–−0.01)**
BI	0.39 (−1.24–2.01)	17.02 (−4.12–38.17)	15.94 (7.22–24.67)**	0.11 (0.03–0.18)*
BBS	0.45 (−0.05–0.95)	11.68 (2.97–20.38)*	10.91 (7.95–13.87)**	0.07 (0.02–0.12)*
Cytokine				
Pro-inflammatory				
IFN-γ	0.14 (−0.03–0.32)	1.24 (−0.56–3.04)	−0.34 (−1.24–0.57)	0.005 (0.0001–0.0107)*
IL-2	−0.06 (−0.07–−0.05)**	−0.09 (−0.23–0.06)	−0.70 (−0.77–−0.64)**	0.0009 (−0.0017–0.0034)
IL-6	0.04 (−0.08–0.16)	−0.44 (−1.71–0.83)	−0.37 (−1.08–0.34)	−0.0028 (−0.0094–0.0037)
IL-17	−0.05 (−0.07–−0.03)**	0.15 (−0.44–0.74)	−2.75 (−2.85–−2.64)**	0.0042 (−0.0004–0.0088)
TNF-α	3.12 (2.37–3.86)**	23.98 (16.51–31.44)**	6.87 (2.57–11.17)*	0.0094 (−0.0091–0.0279)
Anti-inflammatory				
IL-10	−0.08 (−0.11–−0.04)**	−0.44 (−0.98–0.10)	−0.83 (−1.01–−0.65)**	−0.0004 (−0.0043–0.0035)

*P<0.05, **P<0.005 was considered statistically significant after test. HLA: Human leukocyte antigen, NIHSS: National Institute of Health Stroke Scale (score: 0–42), BI: Barthel index (score: 0–100), BBS: Berg Balance Scale (score: 0–56), IFN: Interferon, IL-2: Interleukin-2, IL-6: Interleukin-6, IL-17: Interleukin-17, TNF-α: Tumor necrosis factor-α, IL-10: Interleukin-10, CI: Confidence interval

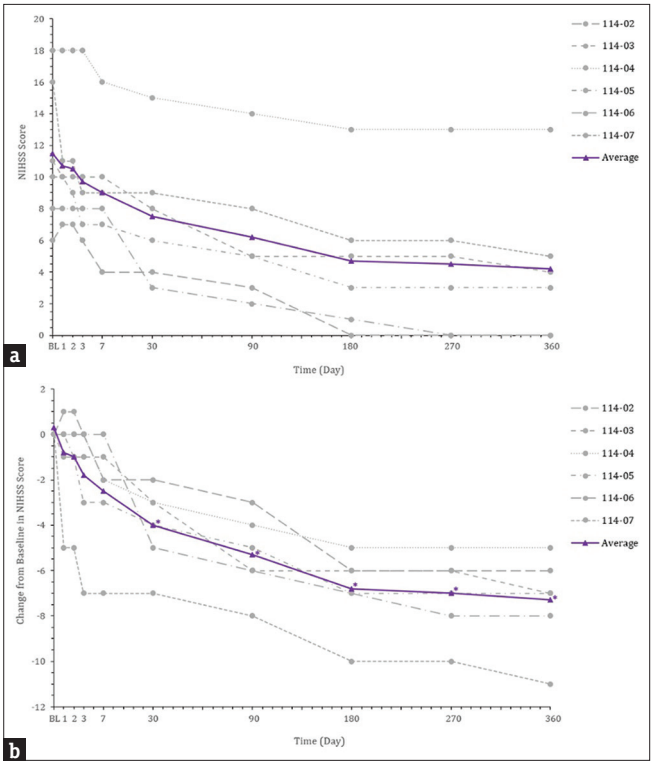

Figure 1: (a) The National Institute of Health Stroke Scale (NIHSS) scores at multiple visits after acute ischemic stroke. (b) Change from baseline in NIHSS scores over time. BL: Baseline, NIHSS: National Institute of Health Stroke Scale (score: 0–42). *Statistically significant compared to baseline, defined as $P < 0.05$

primary concern if applied routinely in the clinical settings [16], particularly regarding the risk of GVHD [17]. We approached this issue with HLA matching to minimize the incidence of GVHD, and in effect, no GVHD was observed in this trial.

For efficacy evaluation, we found significant improvement over time in neurological functions assessed by NIHSS, BI, and BBS scores. The NIHSS is the standard tool to assess the neurological deficits in patients with AIS and higher scores indicated worse performance. The significant improvement occurred at 1 month after treatment and continued throughout the 12 follow-ups. Although there was no control group for comparison, hUCB infusion did not worsen or withhold the course of poststroke recovery by any measure in our study. The control group of a reference study conducted by Shyu *et al*. was a placebo-controlled trial to investigate the granulocyte colony-stimulating factor therapy in AIS subjects [18]. It was found that the subjects in the control group only had a slight change in NIHSS, which were 12.0 ± 2.6 at baseline and 7.7 ± 1.2 at 12 months after AIS. The present study showed a mean change of −7.3 in NIHSS at 12 months of follow-up, which was considered comparable to or even better than the control group in Shyu *et al*.'s study. We demonstrated that the HLA-matching status may contribute to the better recover progress from stroke in the GEE models; however, we interpret the finding with great caution as we have only six patients and further investigation is much needed.

The BI is a functional scale to assess the level of independence of daily living, and all our participants showed functional improvement. The significant change occurred at 3rd month after treatment and continued to the 6th month as expected in the course of stroke recovery.

The BBS is designed to assess the balance function and predictive of the risk of falls. To the best of our knowledge, this is the first study investigating the change in BBS from baseline to post-hUCB infusion in AIS patients. This study showed an improvement from baseline by a mean of 7.7 at 1-month postinfusion. However, the BBS scores of these patients were highly variable with the lowest and highest at 0.0 and 51.0, respectively. Moreover, BBS is more about the lower body parts or mobility function and cannot reflect the neurological function in a comprehensive fashion. We did see some numerical increase in BBS but which did not reach statistical significance. As BBS change of ≥6 at 1-month postinfarction is considered as an important change after

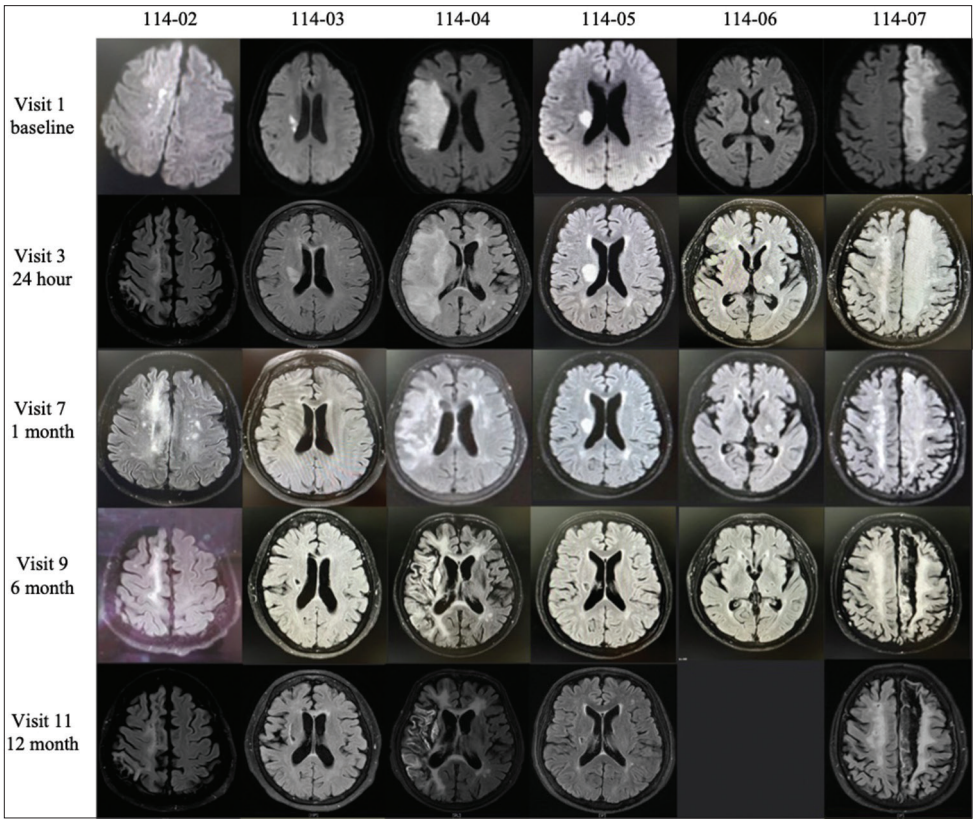

Figure 2: Diffusion-weighted magnetic resonance imaging (MRIs) at baseline and T2-FLAIR MRIs at multiple visits during 12-month follow-ups. Subject no. 114-06 withdrew before visit 11

stroke [19], this study achieved a higher mean change from baseline in BBS score, suggesting an important clinical change in functional balance.

We collected the series of brain MRI from the acute phase to the subacute and recovery phase of stroke, showing the course of initial ischemia along with focal edema, followed by softening, shrinkage, and then loss of brain tissue. The location, severity, mechanism, and complication of each stroke event varies across different individuals, and it is difficult to standardize all these MRI parameters and infer the effect of hUCB infusion in the trial. Nevertheless, the series of MRI scans showed the gradual brain change after stroke and at least there was no deterioration beyond our expectation. For those with infarcts of moderate-to-large size (subjects 114-02, 114-04, and 114-07), the MRI at 12 months after treatment appeared to be preserving more brain tissue in our experience. The observation is unsupported by evidence but worth further confirmation in future trials.

We measured the cytokine levels at multiple time points after stroke. IFN-γ was found to increase over time in the GEE models; however, the inflammatory markers fluctuated without a consistent pattern. The levels of cytokines have to do with age, sex, and other medical conditions. These findings are treated as exploratory and whether this change can be explained by hUCB infusion remains questionable.

The spleen size may shrink in patients with ischemic stroke within 6 h of stroke onset and the process usually continues until the 3rd day, and then, the size gradually increases from the 4th to 8th day [20]. The shrinkage of spleen is associated with the release of pro-inflammatory cytokines (INF-γ, IL-6, IL-10, IL-12, and IL-13), blood leukocytosis, and greater deficits of stroke [21,22]. In this study, patients with AIS were enrolled approximately 6 days after stroke onset, during which the spleen was about to be back to its prestroke state, and therefore, we did not notice any significant change of splenic volume.

The strength of using hUCB cells to treat patients with AIS can be seen from multiple perspectives. First of all, hUCB cells are biologically close to embryonic stem cells, and thus, more immunologically tolerant and of better plasticity, providing a source of stem cells for the ischemic brain. Second, the UCB cell bank has been well established for years with abundance of HLA cell types, making the treatment readily available to AIS patients. Third, we used ABO plus HLA typing to reduce the risk of GVHD, and indeed, we did not have any GVHD event. Finally, the procedure of hUCB infusion followed by mannitol use does not require sophisticated clinical settings or high technical thresholds, allowing the treatment to be feasible in hospitals of different levels.

There are several limitations in our trial though. We included only six patients and their stroke types as well as severity varied. Although the efficacy measurements by NIHSS, BI, and BBS scores were favorable, we could not draw the conclusion that hUCB infusion provided better stroke outcomes in such a small trial. Nevertheless, this trial

demonstrated an acceptable safety profile that almost none of the reported AEs was considered related to hUCB infusion except erythema multiforme and hematuria after mannitol administration. Second, we did not have adequate markers to monitor the dynamics of hUCB cell infusion, and so whether the stem cells arrived at the lesion site or rescued the ischemic cells was not known. The whole process may simply reflect the anti-inflammatory effects of hUCB cells but have nothing to do with cell transplantation. Third, the mannitol use was meant to open the blood–brain barrier for stem cells to reach the lesion site [9], but we are unable to track the actual effect of mannitol following hUCB infusion in the local ischemic area. Given the high proportion of hematuria, the necessity and protocol of mannitol use require further studies.

Conclusions

The single-center, open-label, phase I trial of single-dose hUCB infusion for AIS within 9 days of stroke onset demonstrates an acceptable safety profile and significant neurological improvement in NIHSS, BS, and BBS during the 12-month follow-ups. hUCB infusion may induce anti-inflammatory responses but does not lead to significant changes in cytokine levels and spleen size. Our findings well justify the conduct of phase II trial in future.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Financial support and sponsorship

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Conflicts of interest

Dr. Raymond Y. Lo, an editorial board member at *Tzu Chi Medical Journal*, had no role in the peer review process of or decision to publish this article. The other authors declared no conflicts of interest in writing this paper.

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Supplementary material

Supplemental Table 1: All adverse events

Subjective number	Adverse event number	Adverse event term	Start date	End date	Severity	Hospitalization	SAE
11402	1	Insomnia	June 13, 2019	June 30, 2019	1	No	No
	2	Cold like symptoms	June 21, 2019	July 1, 2019	1	No	No
114-03	1	Erythema multiforme (skin and subcutaneous tissue disorders)	March 19, 2022	March 19, 2022	1	No	No
	2	Insomnia	September 14, 2022	Ongoing	1	No	No
114-04	1	Hematuria	May 14, 2022	May 14, 2022	1	No	No
	2	Urine retention	July 21, 2022	April 12, 2023	1	No	No
	3	Left shoulder anterior dislocation	October 24, 2022	November 22, 2022	1	No	No
	4	COVID-19	2022/10/UK	2022/10/UK	2	No	No
	5	Depression	November 9, 2022	Ongoing	2	No	No
	6	Bedsore	November 9, 2022	Ongoing	2	No	No
	7	Right renal stone	January 13, 2023	March 24, 2023	2	Yes	Yes
	8	Hematuria	January 13, 2023	January 13, 2023	2	No	No
	9	Urinary tract infection	January 30, 2023	Ongoing	2	No	No
	10	Left femoral neck fracture	May 1, 2023	Ongoing	3	No	No
	11	Osteoporosis	May 1, 2023	Ongoing	2	No	No
114-05	1	Hematuria	May 17, 2022	May 17, 2022	1	No	No
	2	Insomnia	May 17, 2022	May 19, 2022	1	No	No
	3	Rhinitis	May 24, 2022	July 21, 2022	1	No	No
	4	Insomnia	July 2, 2022	July 23, 2022	1	No	No
114-06	1	Hematuria	June 7, 2022	June 7, 2022	1	No	No
	2	Hypokalemia	June 20, 2022	June 24, 2022	1	No	No
	3	Hand and foot pain	July 19, 2022	Ongoing	1	No	No
	4	Dermatitis	September 2, 2022	April 10, 2023	1	No	No
	5	Vertigo	October 14, 2022	December 16, 2022	1	No	No
	6	Conjunctivitis	October 25, 2022	Ongoing	1	No	No
	7	UGI bleeding	January 19, 2023	January 19, 2023	3	No	No
	8	UGI bleeding	January 30, 2023	February 4, 2023	3	Yes	Yes
	9	Hyponatremia	May 10, 2023	Ongoing	2	No	No
	10	Abnormal liver mass rule out liver tumor	May 15, 2023	Ongoing	4	Yes	Yes
	11	Cholangitis	May 15, 2023	Ongoing	4	Yes	Yes
	12	Gastric cancer (adenocarcinoma)	May 23, 2023	Ongoing	4	Yes	Yes
114-07	1	Hematuria	June 15, 2022	June 15, 2022	1	No	No
	2	Urinary tract infection	September 3, 2022	September 9, 2022	1	Yes	Yes
	3	Seizure	2023/03/UK	2023/03/UK	2	No	No
	4	Cough	April 3, 2023	May 1, 2023	3	No	No
	5	Fever	April 14, 2023	April 20, 2023	3	No	No
	6	Bladder tumor	April 21, 2023	May 2, 2023	4	Yes	Yes
	7	Pneumonia	April 21, 2023	May 2, 2023	4	Yes	Yes
	8	Hyperplasia of prostate	May 4, 2023	Ongoing	2	Yes	Yes
	9	COVID-19	June 5, 2023	June 10, 2023	2	No	No

CTCAE version 4.03 was used for determining the severity of AE. Severity grade - 1: Mild, 2: Moderate, 3: Severe, 4: Life-threatening. AE: Adverse event, SAE: Serious AE, UGI: Upper gastrointestinal