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Autologous Umbilical Cord Blood Infusion followed by Oral Docosahexaenoic Acid and Vitamin D Supplementation for C-Peptide Preservation in Children with Type 1 Diabetes

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Abstract

We sought to determine if autologous umbilical cord blood (UCB) infusion followed by 1 year of supplementation with vitamin D and docosahexaenoic acid (DHA) can preserve C-peptide in children with type 1 diabetes. We conducted an open-label, 2:1 randomized study in which 15 type 1 diabetes subjects with stimulated C-peptide > .2 pmol/mL received either (1) autologous UCB infusion, 1 year of daily oral vitamin D (2000 IU), and DHA (38 mg/kg) and intensive diabetes management or (2) intensive diabetes management alone. Primary analyses were performed 1 year after UCB infusion. Treated (N = 10) and control (N = 5) subjects had median ages of 7.2 and 6.6 years, respectively. No severe adverse events were observed. Although the absolute rate of C-peptide decline was slower in treated versus control subjects, intergroup comparisons failed to reach significance (P = .29). Area under the curve C-peptide declined and insulin use increased in both groups (P <.01). Vitamin D levels remained stable in treated subjects but declined in control subjects (P = .01). DHA levels rose in treated subjects versus control subjects (P = .003). CD4/CD8 ratio remained stable in treated subjects but declined in control subjects (P = .03). No changes were seen in regulatory T cell frequency, total CD4 counts, or autoantibody titers. Autologous UCB infusion followed by daily supplementation with vitamin D and DHA was safe but failed to preserve C-peptide. Lack of significance may reflect small sample size. Future efforts will require expansion of specific immunoregulatory cell subsets, optimization of combined immunoregulatory and anti-inflammatory agents, and larger study cohorts.

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Keywords

Autologous cord blood; Type 1 diabetes; Vitamin D; Docosahexaenoic acid; C-Peptide

INTRODUCTION

We previously investigated the potential for a single autologous umbilical cord blood (UCB) infusion to augment the natural history of type 1 diabetes in very young children [1–4]. Although our initial effort was not powered to demonstrate efficacy, the apparent safety of autologous UCB infusion as well as the observed postinfusion increase in peripheral blood regulatory T cell (Treg) frequency provided impetus for additional studies. Given our group's emphasis on the potential for combination therapies to provide synergy in attenuating autoimmunity and facilitating the development of low-risk (ie, safe) therapies for young children with type 1 diabetes, we hypothesized that autologous UCB therapy could be successfully augmented by the addition of two agents with well-characterized immunomodulatory properties and potential links to type 1 diabetes pathophysiology, namely, vitamin D and docosahexaenoic acid (DHA) [5, 6]. As such, we sought to perform a follow-up pilot study using this combination approach to document safety and feasibility and to generate data to guide future fully powered studies using autologous cell therapies.

METHODS

Participants

Children with type 1 diabetes older than 1 year of age and for whom autologous UCB was stored were recruited for participation (NCT00873925; FDA IND BB-11918).

Procedures

Potential subjects underwent a baseline 2-hour mixed meal tolerance test to determine eligibility [1]. Subjects with peak C-peptide > .2 pmol/L were randomized 2:1 (treatment-to-control) in an open-label manner. All subjects had blood drawn at baseline for complete blood count, BMP, HbA_{1c}beta cell autoantibodies, vitamin D studies, serum cytokines, and flow cytometry with staining for CD3, CD4, CD8, CD25, CD62L, CD45RA, and FOXP3 performed as previously described [2]. Lipid fractions were obtained from whole blood by a modification of Bligh and Dyer [7]. Fatty acids present were converted to methyl esters [8] and analyzed by gas chromatography (model HP6890; Hewlett-Packard, Wilmington, DE) using a CP-WAX column (Varian, Walnut Creek, CA) with mass spectral detection in electron impact mode.

Subjects randomized to therapy had peripheral blood and an aliquot of UCB shipped to the University of Florida for infectious disease testing, HLA confirmation, and viability screening. Thereafter, the UCB unit of qualified subjects was shipped to the University of Florida and stored until infusion. A detailed description of our UCB infusion protocol was previously published [1].

Subjects received pretreatment with diphenhydramine and acetaminophen. No other preparative therapy was given. Thawed UCB cells were infused through a peripheral intravenous line over 10 to 20 minutes After UCB infusion, subjects randomized to treatment began a regimen of oral vitamin D (2000 IU/day), given as 1 drop of vitamin D3 (2000 IU per drop; Carlson Laboratories, Woodbridge, Ontario, Canada), and oral DHA (38 mg/kg/day) given as 200-mg gel caps (Martek Biosciences Corporation, Columbia, MD).

All subjects returned for follow-up testing (identical to baseline) at 3 months, 6 months, and 1 year.

Statistical Analysis

Because outliers in some variables were expected, nonparametric methods were used for all analyses. Because this is a pilot trial, where Type II errors are as serious as Type I errors, no control for study-wide Type I error was made. Within each treatment group, comparisons for changes from baseline with month 12 were performed via the two-sided Wilcoxon signed rank test. Comparisons for between-treatment changes from baseline with month 12 were performed via the two-sample, two-sided Wilcoxon rank test. Descriptive statistics are given as median (50th percentile) and quartiles (25th and 75th percentile). Sample sizes were not based on a power computation but on constraints of funding and subject availability. For descriptive purposes, $P < .05$ was considered to be significant, but actual P values are provided.

RESULTS

Baseline and longitudinal postinfusion characteristics for all subjects are provided in Table 1. Between April 22, 2009 and August 31, 2010, 15 eligible children with type 1 diabetes were randomized (10 treated and 5 control subjects). Five control subjects (3 boys), median age 6.6 years, received intensive diabetes management alone. Ten treated subjects (8 boys), median age 7.2 years, received a single autologous UCB transfusion. All aliquots of UCB had negative Gram stains, and none grew pathogenic organisms when cultured for virus, bacteria, or fungus. One subject developed a cough during infusion that was assessed as a mild infusion reaction and was treated with 20 mg i.v. diphenhydramine. No other adverse events were observed in association with autologous UCB infusion.

Median time from diagnosis to screening was 119 days in the 10 treated children and 106 days in the 5 control subjects. The median infused total nucleated cell count was 1.1×10^7 cells/kg (0.4×10^7 and 3.9×10^7respectively; N = 10). Median viability was 92.5% (90% and 98%, respectively; N = 10). After UCB infusion, subjects randomized to treatment received 1 year of daily oral supplementation with DHA (38 mg/kg/day) and vitamin D (2000 IU/day). Based on capsule counts, DHA compliance was estimated at 91%.

HbA_{1c} remained statistically unchanged ($P = .4$) in both groups throughout the study, with excellent control evidenced by the mean HbA_{1c} of 7.3% at the 1-year visit. Insulin requirements increased significantly in both groups ($P = .001$), although they did not differ significantly when performing between-group comparisons ($P = .18$).

Among treated subjects, area under the curve (AUC) C-peptide at the time of UCB infusion was 0.30 pmol/mL per 120 minutes (interquartile ratio), whereas control subjects demonstrated baseline AUC C-peptide of 0.55 pmol/mL per 120 minutes. AUC C-peptide declined at all subsequent study visits for both groups compared with baseline ($P < .01$, baseline to 1 year) (Table 1). In addition, although the absolute rate of C-peptide decline was slower in treated (0.23 pmol/mL per 120 minutes) versus control subjects (0.33 pmol/mL per 120 minutes), differences in change of AUC C-peptide between treated and control subjects did not reach significance at 1 year ($P = .29$). Peak C-peptide data demonstrated a similar pattern (Table 1).

When compared with baseline, vitamin D levels were not statistically different at 1 year when evaluating patients who received vitamin D supplementation. However, control subjects demonstrated significant declines in vitamin D levels ($P = .05$), and the intergroup comparison was also significant ($P = .01$).

Fatty acid analysis revealed significant increases among treated subjects treated with DHA (P = .01), docosapentaenoic acid (P =.009), and eicosapentaenoic acid (P =.01) when compared with control subjects. No significant changes were observed in Treg frequency, CD4/CD8 ratio (Table 1), or cytokines and beta cell autoantibodies (data not shown).

DISCUSSION

In contrast to our initial studies of autologous UCB therapy in patients with type 1 diabetes, this protocol required that subjects demonstrate mixed meal tolerance testestimulated C-peptide >.2 pmol/L. Despite the requirement for (at least minimal) endogenous beta cell function, the small sample size in this follow-up pilot study limited our capacity to demonstrate C-peptide preservation after combination therapy with autologous UCB, vitamin D, and DHA.

In addition to the limitation imparted by the relatively small number of study subjects, the capacity to preserve beta cell function may actually be limited by a number of factors. First, an insufficient number of cells that carried regenerative or immunoregulatory capacity may have been transferred to patients with type 1 diabetes. Second, the ongoing autoimmune response in subjects with new-onset type 1 diabetes may contain memory T cells, which are refractive to regulation by Tregs [9], that facilitate the ongoing autoimmune destruction of endogenous or de novo beta cells. Third, vitamin D and DHA, chosen as adjunct therapies largely because of their very-low-risk profiles, may lack the immunomodulatory capacity to provide meaningful treatment in the face of a well-established autoimmune attack.

To address the first issue, efforts are underway to isolate and expand specific cell populations within UCB to augment their therapeutic potential. As proof of concept, a phase I clinical trial is currently underway in adult patients with recent-onset type 1 diabetes using autologous expanded Tregs isolated from peripheral blood (NCT01210664). In terms of the second and third limitations, studies from our laboratory suggest that a therapeutic approach involving transient immune depletion and subsequent induction of immune regulation using a combination of low-dose thymoglobulin and pegylated granulocyte colony-stimulating factor is optimal [10]. As such, we believe that therapies combining transient immune depletion (using thymoglobulin or similar agents) and subsequent infusion of expanded UCB Tregs with or without additional stem cell mobilizing agents may more effectively reset the balance of regulatory and effector T cells in type 1 diabetes. Such approaches will undoubtedly require ongoing discussion regarding equipoise when being considered as options for the treatment of type 1 diabetes in young children.

Although the combination of UCB infusion, vitamin D, and DHA did not demonstrate C-peptide preservation in this underpowered study, the potential of UCB to participate as a meaningful component in type 1 diabetes intervention therapies remains. Given the very small sample size used in this pilot study, we must be careful to avoid the assumption that a lack of statistical significance implies that cord blood therapies are incapable of providing benefit in type 1 diabetes. Indeed, as rates for autologous cord blood banking climb, we suspect that larger studies can and should follow our efforts. Strong scientific rationale for autologous UCB therapies remains. As such, additional efforts to use autologous UCB in the treatment of type 1 diabetes will continue, with emphasis on improved understanding of UCB Treg function and the potential use of expanded autologous UCB Tregs either alone or in combination with other immunomodulatory agents.

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Table 1

Baseline, 6-Month, and 1-Year Characteristics of Subjects Receiving Combination Therapy with UCB, Vitamin D, and DHA Compared with Control Subjects

	Treated (N = 10)				**Control Subjects (N = 5)**				***P* Comparison of Changes: 1 Yr Minus Baseline between Groups**
	Baseline	**6 Mo**	**1 Yr**	***P* for Δ at1 Yr**	**Baseline**	**6 Mo (n = 4)**	**1 Yr**	***P* for Δ at 1 Yr**	
Age, yrs	7.2	—	—	—	6.6	—	—	—	—
HbA_{1c}, %	7.1 (6.3–7.8)	6.8 (6.0–7.4)	7.3 (6.3–7.8)	.89	6.5 (6.4–6.9)	7.2 (6.6–7.5)	7.5 (7.2–7.6)	.63	.13
Insulin dose, units/kg/d	.34 (.23–.46)	.53 (.47–.64)	.63 (.56–.73)	.004	.30 (.29–.32)	.42 (.33–.63)	.65 (.57–.80)	.063	.27
AUC C-peptide, pmol/mL per 120 min	.29 (.26–.47)	.12 (.06–.32)	.10 (.06–.24)	.004	.41 (.29–.77)	.40 (.23–.54)	.14 (.08–.25)	.063	.29
Peak C-peptide, pmol/L	.33 (.24–.52)	.19 (.09–.40)	.12 (.08–.29)	.002	.53 (.37–.86)	.48 (.28–.61)	.17 (.12–.33)	.063	.14
Vitamin D, nmol/L	78 (55–91)	122 (78–140)	96 (78–130)	.19	91 (83–99)	68 (52–120)	62 (42–83)	.063	.010
DHA, μg/mL	22 (19–29)	68 (61–84)	75 (64–79)	.004	18 (14–27)	23 (16–33)	20 (16–24)	.88	.0055
EPA, μg/mL	5.7 (4.4–6.3)	8.7 (6.3–1.1)	8.7 (7.6–10.1)	.012	4.3 (2.8–6.1)	5.4 (3.6–6.1)	5.4 (2.4–6.0)	.63	.031
DPA, μg/mL	13 (12–17)	6.8 (6.0–8.8)	8.4 (6.6–9.5)	.004	12 (10–13)	14 (13–16)	15 (11–16)	.13	.0055
Tregs, %	.47 (.18–1.04)	.21 (.13–.40)	.61 (.33–.75)	.77	.45 (.15–.64)	.20 (.10–.51)	.23 (.20–.31)	.81	.62
CD4/CD8	2.53 (1.67–2.64)	2.21 (1.75–2.63)	2.14 (1.91–2.61)	.82	1.95 (1.92–2.89)	1.85 (1.58–2.65)	1.69 (1.65–1.94)	.063	.066

DPA indicates docosapentaenoic acid; EPA, eicosapentaenoic acid.

Values are medians, with interquartile ratios in parentheses.