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Biochemical and Biophysical Research Communications 321 (2004) 168–171
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Transplantation of human umbilical cord blood cells improves glycemia and glomerular hypertrophy in type 2 diabetic mice

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Received 9 June 2004

Abstract

Recent in vitro and in vivo studies have shown that either animal- or human-derived embryonic stem cells can differentiate into insulin-secreting cells and lower blood glucose levels. However, studies utilizing human umbilical cord blood (HUCB) mononuclear cells to improve blood glucose levels in diabetic animals have received little attention. In this study, we examined the effect of transplanted HUCB mononuclear cells on blood glucose levels, survival, and renal pathology in obese mice with spontaneous development of type 2 diabetes. The results show that injection of HUCB mononuclear cells into orbital plexus of mice caused improvement not only in blood glucose levels and survival rate but also normalization of glomerular hypertrophy and tubular dilatation. Thus, transplantation of HUCB mononuclear cells appears to be another modality of stem cell therapy in diabetes mellitus.

Keywords: Human umbilical cord blood cells; Stem cells; Transplantation; Blood glucose; Glomerulus; Type 2 diabetes; Mice

Islet cell transplantation to improve blood glucose levels has received renewed interest in recent years [1–9]. This procedure requires both adequate islet cell mass and immunosuppression. Therefore, continued efforts are being made to generate new sources of non-pancreating insulin-producing cells. Embryonic stem (ES)-derived cells, either from animals or humans, were found to be one such source [10–20]. These stem cells are pluripotent with differentiation to β-cells that are committed to secretion of insulin. Soria et al. [12] implanted one million mouse ES-derived insulin-secreting cells into the spleen of streptozotocin diabetic mice, and found normalization of blood glucose levels within a week and restoration of body weight in 4 weeks. Shah and Jindal [21] reported reversal of diabetes in the rat by injection of hematopoietic stem cells infected with recombinant adeno-associated virus containing the pre-proinsulin II gene. Stepanovic et al. [22] demonstrated wound healing in type 2 diabetic mice following mouse bone marrow cells injected under skin wounds. Thus, ES cells from different sources can improve glycemia as well as wound healing in diabetes.

In addition to ES-derived and bone marrow stem cells from animals and humans, mononuclear cells derived from human umbilical cord blood (HUCB) were also found to differentiate into cells that improve a variety of disease conditions in animals [23]. In a preliminary study, transplantation of HUCB mononuclear cells into type 1 diabetic mice improved not only their glycemia but also survival [24]. This study prompted us to examine whether a similar improvement in glycemia and survival could be observed in mice with spontaneous development of type 2 diabetes. Furthermore, the influence of these cord blood cells on the pathology of the kidney was examined. The study was performed without any immunosuppression.

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doi:10.1016/j.bbrc.2004.06.121

Materials and methods

Animals. Twenty type 2 diabetic mice (B6.Y-Lep^{ob}), obtained from the Jackson laboratory, were used for the study. Ten mice received 200×10^6 HUCB mononuclear cells retro-orbitally into the venous plexus at the age of 75 days. Ten mice did not receive any cells and served as controls. All mice were weighed every 2 weeks until sacrifice at 392 days of age. Blood for glucose was obtained from the orbital plexus every 4 weeks until sacrifice. Glucose levels were determined by the glucose oxidase method using the reagents supplied by Sigma Chemical (St. Louis, MO). All mice were fed Purina rodent chow 5001 ad libitum and allowed to drink tap water. At the end of the study, the surviving mice were sacrificed under O_2/CO_2 anesthesia and the kidneys were removed. A portion of the kidney was fixed in 10% buffered formalin, 2 μm thick sections were prepared and stained with periodic acid–Schiff for light microscopy. Procedure for the collection and use of HUCB for this study was approved by the Institutional Review Board of New Jersey Medical School, Newark, NJ. The mice were housed in an AAALAC-1 approved animal facility, and the project was approved by the Institutional Animal Review Committee.

Collection and preparation of human cord blood. HUCB samples were obtained from placentas of healthy full-term neonates. Each cord blood sample was collected into a 50 ml sterile polypropylene test tube containing 5 ml of citrate phosphate dextrose as an anticoagulant. The volume collected varied from 20 to 40 ml, and the samples were kept at room temperature until they were sent to the blood bank for storage. The samples were then transferred into a polyolefin blood collection bag (Cryocyte Freezing Container, Baxter Healthcare, Deerfield, IL) that allows gaseous transfer and were stored at 4 °C in a blood bank refrigerator. Donor specimens were combined according to their blood type (ABO). After storage for 10–13 days, units were placed in a 15 ml disposable centrifuge tube and the mononuclear cells were separated from the whole cord blood by Ficoll–Hypaque (Sigma, St. Louis, MO) density gradient centrifugation. The cells were then washed twice with phosphate buffered saline (PBS) and centrifuged for 10 min at 1000 rpm. One milliliter of PBS was added to the pellet for counting. After the viability and counting were determined, the mononuclear cells were centrifuged for 10 min at 1000 rpm, then 0.2 ml of PBS solution was added for final dilution and injection into the mouse (retro-orbital). This process was repeated the next day to bring the total number of mononuclear cells given to the animals up to 200×10^6. This large dose of cells was selected because of previous studies that showed a dose-dependent survival of mice [23].

Statistical analysis. All data are expressed as means ± SD. Student's t test was used to calculate the statistical significance and a P value <0.05 was considered significant. Survival curves were plotted using the Kaplan–Meier method.

Results

Fig. 1 shows body weights in control and HUCB recipient mice. No difference in the body weight was observed between the two groups of mice at the start of the study (75 days of age). The body weight in control mice increased with increasing age. In HUCB recipients, however, the body weight was found to be significantly lower ($P < 0.001$) from 196 to 280 days compared to control mice. This difference was not observed after 280 days of age.

Blood glucose levels in both groups of mice are shown in Fig. 2. Similar to body weights, no difference in blood glucose levels was observed at the start of the study. Blood glucose levels increased with duration of diabetes in control mice, reaching up to 344 ± 1.88 mg/dL at the age of 392 days. In contrast, the blood glucose levels were significantly lower ($P < 0.001$) in transplanted mice. At the time of sacrifice (392 days), the blood glucose levels were 262 ± 1.76 mg/dL.

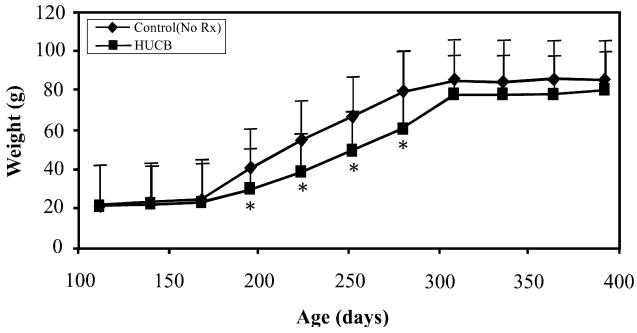

Fig. 1. Body weights (g) at different days of age in control diabetic mice (no Rx) and diabetic mice that received human umbilical cord blood mononuclear cells at different days of age. *Statistical significance at $P < 0.001$. Number of mice at start of study in each group = 10.

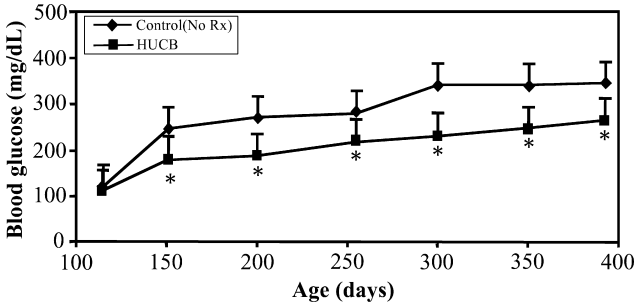

Fig. 2. Blood glucose levels (mg/dL) at different days of age in control diabetic mice (no Rx) and diabetic mice that received human umbilical cord blood mononuclear cells. *Statistical significance at $P < 0.001$. Number of mice at start of study in each group = 10.

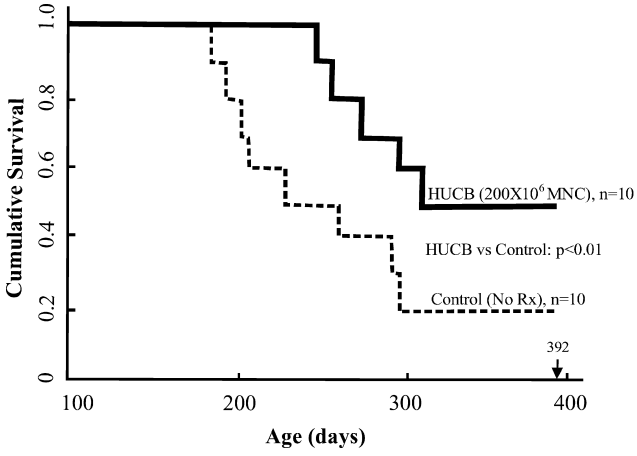

Fig. 3. Kaplan–Meier survival curves for control diabetic mice (no Rx) and diabetic mice that received human umbilical cord blood mononuclear cells.

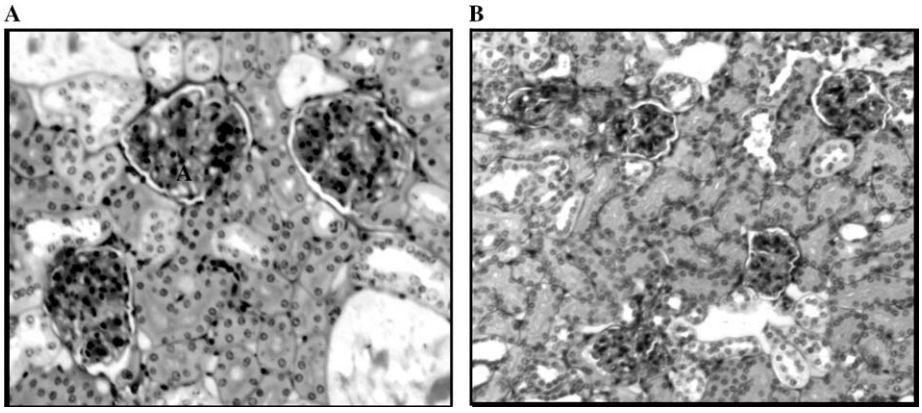

Fig. 4. Photomicrographs of representative glomeruli from control diabetic mice (no Rx) (A) and diabetic mice that received human umbilical cord blood mononuclear cells (B). Note glomerular hypertrophy and tubular dilatation in control and attenuation of these changes in transplanted mice. Periodic acid–Schiff, 100×.

Fig. 3 shows Kaplan–Meier survival curves in control and transplanted mice. All control mice ($N=10$) survived up to 150 days of age; however, only two mice were alive at 392 days. In contrast, five transplanted mice were alive at 392 days of age ($P<0.001$).

Light microscopic structure of the kidney is shown in Fig. 4. Glomerular hypertrophy is clearly evident in control mice, which was attenuated in transplanted mice. Also, tubular dilatation is more evident in control than transplanted mice.

Discussion

This study demonstrates several important observations. First, transplantation of HUCB mononuclear cells improves blood glucose levels and survival in type 2 diabetic mice. Second, both glomerular hypertrophy and tubular dilatation are attenuated by transplantation of these cells; and third, these beneficial effects occurred without prior or concomitant immunosuppression.

Normalization of blood glucose levels within a week was observed in streptozotocin diabetic mice following splenic transplantation of ES-derived insulin-secreting cells [12]. However, this blood glucose-lowering effect was not observed in 40% of the mice 12 weeks following transplantation. In our study, we were able to observe lowering, but not normalization, of blood glucose levels until the end of the study. Thus, transplantation of HUCB mononuclear cells appears to be a better approach for continued improvement of blood glucose levels in diabetic mice.

The observed 80% decrease in survival in untreated mice is not unexpected. The improved survival in transplanted mice appears to be related to maintenance of moderate blood glucose levels.

To our knowledge, no study has addressed the implication of stem cell therapy on kidney disease in diabetic animals. Renal hypertrophy is a well-documented phenomenon in type 1 and possibly type 2 diabetic animals and human beings [25,26]. In our study, glomerular hypertrophy and tubular dilatation were observed in control mice, and both these abnormalities were attenuated by transplantation of HUCB mononuclear cells. This attenuation occurred in the presence of moderate hyperglycemia, suggesting that HUCB mononuclear cells may have a different mechanism to normalize renal growth.

In conclusion, transplantation of HUCB mononuclear cells into type 2 diabetic mice improves not only blood glucose levels and survival, but also glomerular hypertrophy without prior or concomitant immunosuppression. The mechanisms by which these improvements occurred remain unknown.

Acknowledgments

This study was supported by the Abraham S. Ende Research Foundation. We wish to thank Dr. Milton Ende, Ms. Adaline Wood, Ms. Yolanda Mohammed, and the Obstetrics staff of the Southside Regional Medical Center, Petersburg, Virginia. We also thank Susan Delia and Sunil Kuppasani for their help with histology of the kidney.

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