Experimental paradigm

Preliminary uninjured mice performed neurobehavioral tests, cell homing, anatomical damage, expression of neural inflammation markers and ELISA experiments were performed at the time points indicated.

Mice were iv transplanted with 1x10⁶ MNC (triangle) or PBS (white circle) one day post-trauma (arrow). Neurologic disability was evaluated for the extent of recovery (NSS). (B) Representative FACS analysis of HUCB-derived CD45⁢⁺ hematopoietic cells immunophenotype profile after magnetic separation using CD45 marker. (C) Representative SEM image of HUCB-derived MNC.

(A) Mice were iv transplanted with 1x10⁶ MNC (triangle) or PBS (white circle) one day post-trauma. (B) Representative FACS analysis of HUCB-derived CD45⁺ hematopoietic cells immunophenotype profile after magnetic separation using CD45 marker. (C) Representative SEM image of HUCB-derived MNC.

(A) Mice were iv transplanted (1x10⁶) with CD45⁺ cells (triangle), CD45⁻ cells (square), MNC treated with anti-CD45 antibody (diamond) or with PBS (white circle) one day post-trauma (arrow). Neurologic disability was evaluated for the extent of recovery (NSS).

(A-C) Mice were iv implanted and neurologic disability was evaluated for the extent of recovery (NSS). (A) 1x10⁶ (square) or 1x10⁵ (triangle) MNC, PBS (white circle) one day (arrow) post-trauma. Insert: neurologic severity score (NSS) evaluation. (B) 1x10⁵ MNC one day (black arrow; triangle) or 8 days (striped arrow; square) post-trauma; PBS one day (white circle) or 8 days (white triangle) post-trauma. (C) 1x10⁵ MNC at a single dose one day (black arrow; triangle) or at a double dose one day (black arrow) and 8 days (striped arrow; square) post-trauma; PBS (white circle) one day post-trauma.

(A) Hemorrhage lesion area (mm²) was calculated upon head non-invasive NIR scanning, at different time points: white bars-PBS, black bars-MNC. Insert: Typical NIR fluorescent micrographs of mice heads taken 7 days after MNC (right) or PBS (left) transplantation. (B) Twenty one days post-trauma, mice were sacrificed and brain slices were stained with Giemsa. Representative frontal (upper panel) and parietal (lower panel) brain sections of CD45⁺ MNC and PBS transplanted mice. Red marked area is the extrapolated missing brain piece in the injured area (arrow). Evaluation of lesion volume was calculated as percentage of the area of contralateral hemispheric tissue. *p<0.05 as compared to PBS group.

(A-D) Mice were iv transplanted one day post-trauma with 1x10⁵ (diamond) or unlabeled (MNC-unlabelled) MNC or with PBS (CHI vehicle). Sham animals, without CHI treatment, also transplanted with labeled MNC (CHI MNC-NIR-TAG⁺) or untreated (CHI MNC) and served as control groups. Representative micrographs of mice heads (B) and isolated brains (D) 5 h after labeled MNC (right) or PBS (left) transplantation. Lesion area marked in a circle. (B) Signal intensity was calculated as a ratio to background fluorescence and is presented in arbitrary units (A.U.). *p<0.05 as compared to PBS group.

(A) Mice were iv transplanted (1x10⁶) one day post-trauma with MNC (b), CD45⁺ cells (c) or with PBS (a). Two hours later mice were sacrificed, brains were removed and stained with anti-human CD45 antibody (green) and DAPI (blue). CD45⁺ group was also stained with anti-human nuclear antibody (red) (d).

(A-D) Mice were iv transplanted (1x10⁶) one day post-trauma with MNC, CD45⁺ cells, CD45⁻ cells or with PBS. Three white bars and 35 days (black bars) post-trauma, mice were sacrificed, whole brains were removed. Isolateral and contralateral cortex tissues were separated and homogenized for protein extraction. 

(B) Mice were iv transplanted (1x10⁶) one day post-trauma with MNC, CD45⁺ cells, CD45⁻ cells or with PBS. Three white bars and 35 days (black bars) post-trauma, mice were sacrificed, whole brains were removed. Isolateral and contralateral cortex tissues were separated and homogenized for protein extraction. 

Neurotherapeutic effect of cord blood derived CD45⁺ hematopoietic cells in mice brain after closed head injury

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Abstract

Traumatic brain injury (TBI) causes disability and death. Cell therapy by human umbilical cord blood (HUCB) transplantation has shown promising results in animal models of TBI and is under evaluation in several clinical trials. HUCB contains different stem cell populations, but only mesenchymal stem cells were evaluated for therapy of TBI. Here we confirm and further extend the characterization of the neurotherapeutic effect of HUCB-derived mononuclear cells and demonstrate for the first time, that HUCB-derived CD45 positive (CD45⁺) cell subset reduced the neurobehavioral deficits which typically occur in a mouse model of closed head injury (CHI). Using neurobehavioral scoring, a CD45⁺ cell population was obtained which was characterized by expression of CD45 and CD11b (96-99%). Intravenous transplantation of these cells one day post-trauma resulted with a significant therapeutic effect observed up to 35 days as evaluated by neurological score reflecting neurobehavioral improvement. This therapeutic effect was in a direct correlation with the decreased lesion volume. Treatment of the cells with anti-CD45 antibody decreased the beneficial neurotherapeutic effect of the cells. CD45⁺ cells were detected by immunohistochemistry at the site of brain injury 2 h after transplantation. Preliminary experiments suggest attenuation of astroglia and microglia activation by decreased immunoreactivity of glial fibrillary acidic protein (GFAP) and allograft inflammatory factor 1 (AIF/Ilbα), at the site of brain injury, 20 days after CD45⁺ cells transplantation. These findings indicate the neurotherapeutic potential of HUCB-derived CD45⁺ cell population in a mice model of brain trauma and propose their use in new clinical settings.

Conclusions

Transplantation of HUCB-derived CD45⁺ hematopoietic cells reduced neuronal deficits and brain damage in CHI mice.

HUCB-derived cells induced this neurotherapeutic effect upon homing to the site of brain injury.

Preliminary evidence of HUCB-derived CD45⁺ ability to attenuate neural inflammation at the brain injury area.

Cells administration as late as 8 days post-injury is as efficient as their administration 1-day post-injury.

HUCB-derived CD45⁺ cells are proposed for cell therapy of patients with brain trauma.

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