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DONOR-CELL ENGINEERING WITH GLYCOGEN-SYNTHASE-KINASE-3 BETA INHIBITOR-LOADED SYNTHETIC NANOPARTICLES ENHANCES LONG-TERM HAEMATOPOIETIC ENGRAFTMENT FOLLOWING IN UTERO TRANSPLANTATION

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Aim of Study: The aim of the present study was to determine whether donor cell engineering with glycogen-synthase-kinase-3 beta (GSK3β) inhibitor-loaded nanoparticles enhances long-term haematopoietic engraftment following in utero transplantation (IUT).

Methods: GSK3β inhibitor-loaded multilamelar lipid vehicles (MLV-CHIR99021) were synthesized and conjugated to the cell membrane of donor bone-marrow mononuclear cells (MNC; B6-GFP+ / B6-CD45.1+). IUT was performed in Balb/c mice at E14 (107 MNC/fetus). Donor cell haematopoietic chimerism was assessed in blood for up to 24 weeks following birth by flow cytometry (% GFP+ within CD45+). To investigate whether MLV-CHIR99021-"decoration" enhanced MNC repopulating function via a pseudo-autocrine mechanism, we performed "competitive" IUT using 1:1 mixtures of conjugated/unconjugated GFP+ and CD45.1+ MNC (engraftment assessed blood at 4 weeks; % GFP+ and CD45.1+ within CD45+). Statistical analysis was performed using 1- or 2-way ANOVA with Bonferroni tests.

Results: Sustained (7-day) in vivo release of the inhibitor in MLV-CHIR99021 animals (MLV-CHIR99021-"decorated" MNC; dose: 1.3*10-7μg/cell or 4mg/kg/fetus) resulted in increased donor cell engraftment at 4 weeks of age (mean±SEM; 52.9±2.8%) that was 3 times greater to that observed in control (MNC only; 15.4±1.4%; p<0.0001) and bolus-CHIR99021 (CHIR99021 4mg/kg/fetus bolus with donor MNC; 10.5±2.0%; p<0.001) animals. This was maintained for up to 24 weeks of age (48.5±3.1%; p<0.0001), with multi-lineage hematopoietic differentiation. In our competitive IUHCT, we observed enhanced engraftment of only MLV-CHIR99021-conjugated donor cells (Figure A, B); this is consistent with a competitive advantage of "decorated" cells and a pseudo-autocrine mechanism of action of CHIR99021 released by MLV.

Conclusion: Cell engineering with GSK3β inhibitor-loaded nanoparticles enhances hematopoietic engraftment of MNC following IUT. Prolonged retention of the biodegradable nanocarriers on cell surfaces enables sustained CHIR99021 release and pseudo-autocrine bioactivity. Conjugation of drug-loaded particles directly to donor cells allows targeted augmentation of their repopulating function, and could markedly increase the therapeutic potential of IUT.