Evaluation of the potential therapeutic use of immature stem cells in a canine model for Duchene muscular dystrophy

Ambrosio CE², Kerkis I¹, Martins DS², Kerkis A³, Miglino MA² & Zatz M⁴

¹Laboratorio de Genetica, Instituto Butantan, Sao Paulo, SP, Brasil
²Departamento de Cirurgia da Faculdade de Medicina Veterinaria da Universidade de Sao Paulo, SP, Brasil
³Genetica Aplicada, Atividades Veterinarias LTD, Sao Paulo, SP, Brasil
⁴Centro de Estudos do Genoma Humano, Departamento de Genetica e Biologia Evolutiva, Universidade de Sao Paulo, SP, Brasil

Published on 16 May 2007

Introduction:

Duchene muscular dystrophy (DMD) is a most severe form of muscular dystrophy, which is inherited as a sex-linked recessive trait and affects 1/3500 of newborn males. Molecular genetic studies indicate that DMD is the result of mutations in the huge gene that encodes dystrophin. In order to confirm the results obtained from mouse model, which did not provide clinical sings of the disease, it has been proposed that muscular dystrophy in the golden retriever dog may be homologous to human to clinical trials. To compare two types of adult stem cells, Umbilical Cord CD 34+ and Dental Pulp Stem Cells (IDPSC), as potential multipotent stem cells for cell therapy use, by the evaluation of their skeletal myogenic potential, migration ability and capacity to restore dystrophin function in skeletal muscle cells of dystrophic young dogs.

Methods:

Each cell type was analyzed according to their morphology, ultrastructure (confocal, immunohistochemistry and TE microscopy), and cell culture ability. In vivo tests were carried out to analyze engraftment features after infusion of Dil-labeled cells, without any immune suppression, either into the femoral artery or by intramuscular injection of 30-days-old dystrophic dogs. After 60 days, biopsies were taken for tissue immunostaining with anti-IDPSC antibody developed in our laboratory. Clinical trials were made performing critical analyses of disease evolution.

Results:

By TEM, canine umbilical cord progenitor cells had an immature cell structure, differing from all primitive blood components. Cell cultures showed poor proliferation. Also, cells, obtained by density solution and magnetic separation, were used for injections into the biceps femuralis or the femoral artery. After 60 days, tissue biopsies failed to demonstrate the presence of dystrophin either by immunohistochemistry or by protein blotting. Conversely, the analysis of tissue biopsies of animals injected with IDPSC showed denser cell engraftment, as indicated by both the presence of Dil-stained cells and anti-IDPSC
antibody positive labeling. Clinical aspects were considered relevant, with the demonstration of significant differences depending on the route of injection and cell type.

**Conclusion:**

The efficacy of arterial injection of pulp dental cells to treat muscular dystrophy demonstrated that canine multipotent stem cells have great potential for cell therapy, promising to become a new trend for therapeutical approaches aiming muscular dystrophy.