

BIODISTRIBUTION OF HUMAN IMMATURE DENTAL PULP STEM CELLS FOLLOWING IN UTERO TRANSPLANTATION IN CANINE MODEL

Ana Luisa Reginato MS, Departamento de Cirurgia da Faculdade de Medicina Veterinária da Universidade de São Paulo, Av. Prof. Dr. Orlando Marques de Paiva, 87 CEP 05508 270 - Cidade Universitária São Paulo/SP – Brasil. Tel/Fax: +55 11 30917690, anareginato@hotmail.com

Reginato Ana L.¹ MS, Fernandes Renata A.¹ BS, Faro Cristina B.A.² MD, BS, Wenceslau Cristiane V.¹ MS, Lizier Nelson F.³ BS, Braga Patrícia C.B.B.⁴ PhD, Kerkis Alexandre⁵ PhD, Miglino Maria A.¹ PhD, Kerkis Irina³, PhD.

¹ Departamento de Cirurgia da Faculdade de Medicina Veterinária da Universidade de São Paulo (USP) ² Universidade Estadual de Campinas, ³Laboratório de Genética, Instituto Butantan, ⁴Escola de Artes Ciências e Humanidades da USP, ⁵CELLTROVET, Genética Aplicada, São Paulo, SP, Brasil.

Introduction In utero cell transplantation is a method of disease treatment based on injection of donor cells into a fetus during gestation. The early gestational fetus is immunologically immature, and is susceptible to tolerance induction by foreign antigens. Previously we reported isolation of human immature dental pulp stem cells (hIDPSC) from baby teeth. They showed expression of mesenchymal and embryonic stem cells markers as well as presented multilineage differentiation *in vitro* and *in vivo* into three embryonic layers. In present study we aimed at in-utero transplantation of hIDPSC into canine fetuses in order to analyze their biodistribution and differentiation within the tissues and organs providing a safe approach for cell therapy.

Methods All experimental procedures were approved by the Ethical Committee of the School of Veterinary Medicine and Animal Science of São Paulo University and were performed under general anesthesia. Human IDPSC of male origin established and described previously were used (Kerkis et al., 2006). Six millions (10^6) of undifferentiated green fluorescent protein (GFP) - positive hIDPSC were transplanted following laparotomy and intraperitoneal injection under intra-operative ultrasound control into five fetuses at the 45 days of gestation. Five fetuses, which did not receive hIDPSC, were used as a control. During seven days ultrasound analyses were performed daily. Further, ovarian hysterectomy was performed and the fetuses were collected. Tissue samples from each organ were isolated and fixed in 4% paraformaldehyde or cryopreserved. Biodistribution of hIDPSC within the organs and tissues were analyzed on cryosections (5 μ m) under Confocal Microscopy.

Results Transplantation procedure was well accepted by the fetuses and the mother and we did not observe any hemorrhage or intra-abdominal liquid accumulation. The hIDPSC, which express GFP protein were found in spleen, muscle tissues, liver, gut, blood vessels and brain. In thoracic muscle hIDPSC were localized in blood vessel in tunica externa. In jejunum (gut) the cells contribute into epithelium of the mucosa (cover villi). In cerebellum we found hIDPSC in the molecular layer presenting morphology of cerebellar Purkinje cells. In all tissues hIDPSC presented cluster localization. Interestingly, although hIDPSC were injected into the fetuses intraperitoneally their presence was evidenced in placenta, especially in muscle layer (tunica media) of placenta artery. Our morphological data indicate that the cells undergo differentiation after homing.

Conclusion Present study showed that xenotransplantation and in utero application of hIDPSC was safe. Cluster biodistribution of hIDPSC following in utero transplantation into canine fetuses in majority of tissues were observed. These cells were able to cross placental barrier presenting homing in placental vessels.