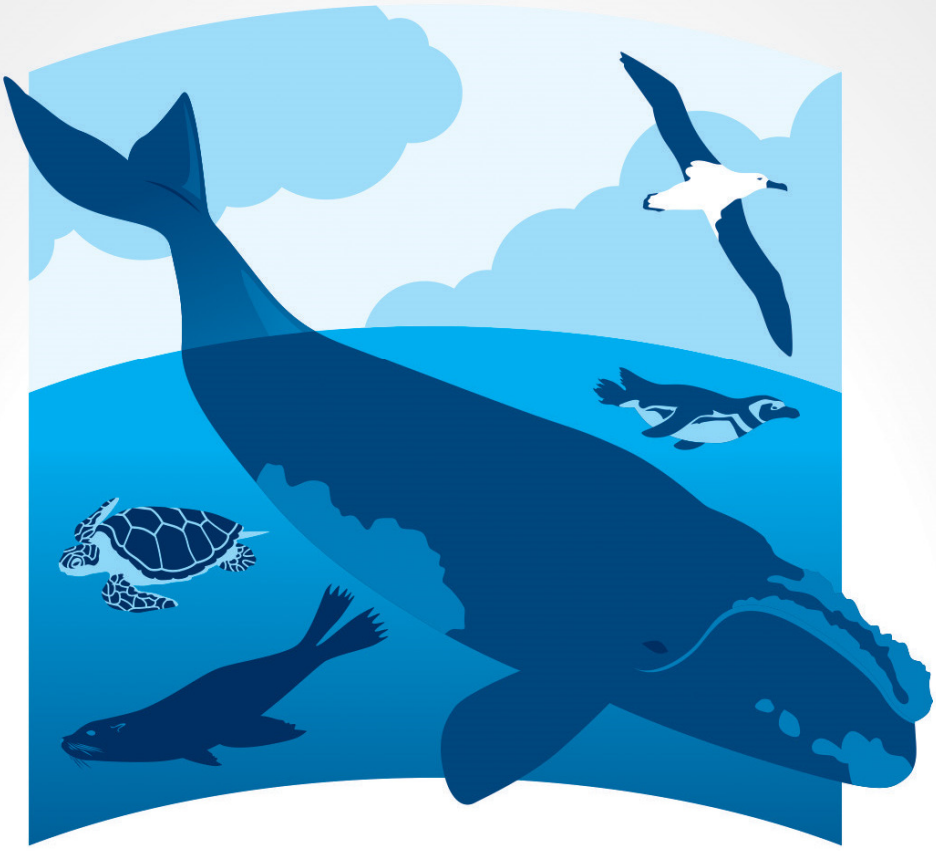

4º CONGRESSO LATINO-AMERICANO DE REABILITAÇÃO DE FAUNA MARINHA

DE 03 A 06 DE SETEMBRO - FLORIANÓPOLIS - SC - BRASIL

LIVRO DE RESUMOS

ELECTROACUPUNCTURE AND INTRATHECAL TRANSPLANTATION OF HETEROLOGOUS ADIPOSE-DERIVED MESENCHYMAL STEM CELLS FOR TREATING SPINAL CORD INJURY IN A LOGGERHEAD SEA TURTLE (CARETTA *CARETTA*)

Daphne Wrobel Goldberg[1], Marina Landim Alvarenga[2], Lucas Feijó Bianchini[3], Daniela Gurgel da Costa[4], Rodrigo Vanucci[4], Daniel Wagner Rogerio[5], Camila Cegoni[5], Juçara Wanderlinde[5]

1 - Programa de Pós-Graduação em Ciência Animal Universidade Estadual de Londrina – UEL; 2 - Omics Biotecnologia Animal; 3 - FisioPet São Francisco - Centro de Reabilitação e Bem Estar Animal; 4 - Fundação Pró-Tamar/Ubatuba; 5 - Fundação Pró-Tamar/Florianópolis; 1 Email: daphwrobel@gmail.com

Introduction

The major goal of rehabilitating injured sea turtles is to nurse these animals back to health and release them back into the sea. Sea turtles are vulnerable to most anthropogenic effects, and impact traumas (e.g. boat collision and propeller impact) are a common cause of severe injuries and death among populations. Individuals swimming or feeding at or just beneath the surface of the water are particularly prone to vessel strikes, which can lead to severe lesions, such as spinal cord injury (SCI). SCI is one of the most severe complications of spine lesions and may cause irreversible damage to neural tissues. The major challenge in the treatment of SCI is achieving axonal regeneration. Recent studies have demonstrated the therapeutic potential of mesenchymal stem cells (MSCs) associated with electroacupuncture (EA) to treat nervous system diseases, especially due to their regenerative, anti-inflammatory and immunomodulatory properties, modulating the microenvironment and facilitating axonal regeneration. MSCs are undifferentiated stromal cells that can be isolated from different tissues, being the most commonly sources the bone marrow and the adipose tissue. These cells have the potential to differentiate into bone, cartilage, fat, tendon, vascular endothelium and hematopoietic tissues. On the other hand, EA is a modern type of acupuncture that involves inserting needles and providing stimulation through electric pulses. According to literature, EA improves the local microenvironment of the spinal cord, reduces edema, induces the repair of neural function and inhibits tissue necrosis. The main goal of this study was to isolate and culture adipose tissue stem cells from a healthy loggerhead turtle (*Caretta caretta*), to treat hind flipper paralysis due to a propeller injury, associating this treatment with electroacupuncture.

Material and Methods

On 7 January 2017, an adult *C. caretta* was rescued by Projeto Tamar (Brazilian sea turtle conservation program) after stranding in Lagoa da Conceição, Florianópolis municipal district, in Santa Catarina State, Brazil. On admission, the animal measured 87 cm curved carapace length, and weighed 73 kg. The turtle was lethargic, emaciated and presented parallel slicing wounds on the caudal part of the carapace and hind limbs. Neurological examination revealed bilateral hind limb paralysis. The turtle underwent a CT scan to evaluate the full extent of the damage, which revealed lumbosacral spine subluxation with vertebral endplates remodeling. EA was incorporated into an intensive physical rehabilitation programme to manage pain, to stimulate the nerve recovery and to reduce inflammation. Location of the EA points was extrapolated from canine acupuncture charts, using the following points: ST36, GB34, BL40 and BL60. After careful consideration, and due to persistent symptoms, an alternative treatment using adipose-derived mesenchymal stem cells (ADMSCs) was considered. We collected subcutaneous adipose tissue from a healthy female donor, held in captivity at TAMAR´s Visitor Center, in Ubatuba, São Paulo. The turtle was anesthetized, and bilateral skin incisions were made along the inguinal area. Fat tissue samples were sent to OMICS Biotechnology Animal Laboratory. For transportation (6 hours), the material was

placed in an antibiotic/antifungal solution containing a combination of penicillin, streptomycin, amphotericin and amikacin (AAS), to avoid bacterial and fungal contamination. In the lab, tissue samples were placed in Trypsin (TrypLE Express ®) overnight. After enzymatic digestion, the solution was filtered, diluted 1: 1 in DMEM F12 with 20% fetal bovine serum (FBS), antibiotic and antifungal and centrifuged at 1500 rpm for 10 min. The cell pellet was re-suspended in two 25 cm^2 culture bottles/group. Culture was performed in DMEM F12 with 20% FBS, antibiotic and antifungal at 28°C and 5% CO^2 in air. Culture flasks were observed every four days to assess cell growth. After 15 days of culture fibroblastoid-like cells adherent to the plastic were observed. After 30 days of culture the cells reached confluence and were submitted to first passage with 97,5% of cell viability according to Trypan Blue staining. 10 x 106 Heterologous ADMSCs were administered intrathecally through the turtle´s tale, without prior sedation. The remaining cells were cryopreserved in liquid nitrogen in the lab for future applications.

Results and Discussion

After six EA sessions, the turtle gradually recovered cloacal sphincter tone, however, hind limbs were still paralyzed. Because of the persistent symptoms, ADMSCs therapy was tested. After four ADMSCs applications and 24 week of EA sessions, the turtle started showing mild improvements in neurological function such as, return of sensibilityand subtle movements of the hind flippers. The treatment is still in process and we hope to get better results. MSCs transplantation and EA stimulation are promising therapies for SCI. Both treatments have the potential to improve neurological deficits while reducing brain injury, and their combination enhances endogenous neurogenesis through the activation of trophic factors in the brain, leading to the recovery of neurological function. To our knowledge this is the first report of isolation and culture of adipose-derived mesenchymal stem cells obtained from *Caretta caretta*, as well as the first treatment using stem cells for SCI in turtles.

Acknowledgments

The authors thank Omics for their technical assistance with the stem cells.