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| Harnessing of Mesenchymal Stem Cells For Treatment and Rehabilitation of Inducing Tendonitis in Equine Species | | | | Thesis Title |
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| This study was planned to assess the potency of adipose derived-mesenchymal stem cells (AD-MSCs) to improve the repair and regeneration of superficial digital flexor tendons (SDFTs) in equine species after experimentally induced-tendonitis, via collagenase injection in tendon core.  Forty adult donkeys (males and females), aged (2-3) years, were used. The experiment was divided into two stages as follows:  **1- First stage:** this stage was carried out on eight donkeys scheduled for harvesting and isolation of MSCs from gluteal subcutaneous adipose tissue (A.T). Surgery was done under the effect of local subcutaneous infiltration of 2% lidocain (1mg/kg B.W) at the site of the incision.  AD-MSCs were cultured in a high quality culture medium (Dulbecco's Modified Eagles Medium (DMEM)) supplemented with 10% of fetal calf serum (FCS) and expanded by three passages, in order to obtain a sufficient number of MSCs for transplantation. This is the first trail of isolation of MSCs from A.T by simple facilities in Iraq.  Immunophenotypic analysis of AD-MSCs indicated that most of these cells express positive response to cluster differentiated-90 (CD90+) and CD105+, and negative for CD34-, CD45-.  The differentiation of MSCs was directed towards the tendon-like cells (tenocytes) by adding bone morphogenetic protein-12 (BMP-12), at a concentration of 50ng/ml of culture medium.  The same animals which used for harvesting of A.T were harnessed to prove of collagenase capability to induce tendonitis in SDFT of fore limb, via intratendenous injection of collagenase, 0.5ml (0.25%) solution in the middle third of SDFT, with the help of ultrasound guidance. The injection was a accomplished under the effect of deep sedation (acepromazin maleate) in a dose of (0.1mg/kg B.W, I.M). Some animals, in equal number were left for (48) hours, post-collagenase injection (acute tendonitis) and other for (14) days (chronic tendonitis).  **1- Second stage**: this stage was carried out on (32) donkeys allocated randomly into four groups, two for control and two for treatment (eight animals / group). All animals subjected to collagenase injection to induce tendonitis in the SDFT of right fore limb. After that, treatment of tendonitis was performed as follows:  III  ii   1. **Control groups**: involved (16) donkeys, divided equally into two groups (A and B). Animals of group (A) were injected with a single dose of 1ml of DMEM free serum after (48) hours, whereas, animals of group (B) were injected with the same medium after (14) days post-inducing tendonitis. 2. **Treatment groups:** also included (16) donkeys, allotted into two equal groups (C and D). One ml (106) of allogeneic AD-MSCs was implanted in core lesion of the injured tendon, (48) hours in group (C), and 14 days post-inducing tendonitis in group (D).   All experimental animals followed-up for (16) weeks, clinically, ultrasonographically and histopathologically.  Post-collagenase injection and during clinical examination, we trapped minor non-specific secondary health problems represented by pain on palpation, local hyperthermia, swelling and lameness. After treatment with MSCs, the above mentioned complications were subsided gradually with advancement of time and totally disappeared after (45) days, while, they remained till the end of the study in the control groups.  Ultrasonographies were performed (48) hours and (14) days post-collagenase injection, to improve the efficiency of this enzyme in destruction of collagen fibers of the tendon. Images showed presence of diffuse or uniform hypo to anechoic areas at the site of injection, (48) hours post-collagenase injection which reached its peak on day (14). In treatment groups, ultrasound images were taken bimonthly to follow the echogenicity and orientation of the collagen fibers, which revealed an increased echogenicity and regular orientation of tendon fibers. By the end of the study, the echogenicity grade was (1), and the grades of fibers alignment ranged from (0 to 1) in treatment groups. In control groups, images revealed presence of hypoechoic areas with randomly arranged collagen fibers, at the end of study, and the echogenicity with fibers alignment were graded as (2).  Histopathological sections from the tendons of animals subjected to collagenase reflected severe destruction of tendon fibers leaving irregular large cavities in tendon core together with polymorphic inflammatory and mononuclear cells infiltration, especially on day (14). In treatment groups, histopathology was performed at (8) and (16) weeks post-MSCs implantation, the fascicular tissue showed a relatively normal appearance (tendon like-structure) replacing the injured area, especially in chronic form compared with acute ones. In contrast, sections obtained from control groups revealed infiltration of inflammatory cells and fibro-cartilaginous tissue formation at the end of study.  IV  In conclusion, the implantation of AD-MSCs in the injured tendon leads to improvement of tendon healing and regeneration from histological and physiological aspect prior to injury. While, the damaged tendons of control groups were healed by scar tissue formation. In addition, the present study hypothesizes that allogeneic AD-MSCs after *in vivo* transplantation into the injured tendon do not provoke an adverse immune response. These results will encourage veterinarians to use stem cells as a novel strategy for treatment of tendonitis in equine species without hesitation. | | | | Abstract |