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MESENCHYMAL STEM CELLS CONTRIBUTE TO STRUCTURAL AND MORPHOLOGICAL RECUPERATION OF PROLAPSED ANTERIOR VAGINAL WALL: IN VITRO STUDY

Hypothesis / aims of study

Pelvic organ prolapse (POP) is a prevalent and growing condition. Currently available therapies have failed to be 100% curative, with recurrence rates of up to 30% for surgeries that use native tissues and with high complication rates when nonabsorbable prosthetic meshes are used (1). Multiple POP causal factors have been identified, being pregnancy and vaginal delivery the most important ones. However, there is growing evidence that connective tissue's structural abnormalities might as well predispose women to POP. These abnormalities refer to a change in the relationship between elements of the extracellular matrix (ECM), like Collagen I, III and V, a decreased production of elastin, an alteration in the metalloproteinases and their Inhibitors ratio and changes in Fibulin 5 levels. It would be therefore highly desirable to develop a therapy for genital prolapse that does'n t uses synthetic components and let the tissues recover their original morphofunctional properties. We propose that the ideal tool to achieve this goal are stem cells, specifically mesenchymal stem cells (MSCs). These have the potential of self-renewal and differentiate, both in vitro and in vivo, into mesodermal cells such as osteoblasts, chondrocytes, tenocytes, adipocytes, muscle fibers, cardiomyocytes, stromal and hematopoietic precursors. It has been recently suggested that MSCs, without the need to differentiate into any particular lineage, may contribute to the regeneration of various tissues by the modification of both the ECM and the production of trophic factors that promote proliferation, differentiation of local precursors, neovascularization and even limiting the immune response (2).

So, based on the above, we hypothesize that allogeneic MSCs implantation directly into vaginal wall fascia from women with pelvic organ prolapse may return them to their original morphofunctional qualities.

The aim of this study is to evaluate if MSCs inoculated directly into vaginal wall explants of women with POP could help them to recover their original morphological features.

Study design, materials and methods

17 women, 11 with POP and 6 without POP, mean age 50±6.4 and 61.2±10.9 years old respectively were adressed. They donated prolapsed or non prolapsed anterior vaginal wall fascia during benign gynecologycal surgeries.

Fascial explants from women with prolapse were fractionated under sterile conditions in 6 equal pieces, and each one was placed in culture in a 99%humid atmosphere with 5% CO2 at 37ºC. They were inoculated with $1x10^4$ MSCs (3 pieces) or Vhcl (3 pieces) two hours after they were placed in culture.

Fascial explants from women without prolapse were fractioned in 3 equal pieces and received the same treatment as above.

After seven days, the samples were analized for: I) Histology: Hematoxilin Eosin (H&E), Masson´s Trichrome Stain (TM) and Van Giesson-Elastic (VE), II) Messenger RNA expression by real time Protein Chain Reaction (RT-PCR) and III) Immunohistofluorescence by Confocal Microscopy analysis for proteins Collagen type I and Fibulin 5

Results

I. Histology: Cell abundance was not altered in both non prolapsed and prolapsed fascia with HE. Masson´s Trichrome Stain (TM) showed a small, fusiform shape cell population at the basal connective tissue line when observed in prolapsed fascia inoculated with MSCs. These cells were not observed in prolapsed fascias inoculated with Vhcl. There also is an increase in stained collagen fibers around these cells. Van Giesson-Elastic (VE) staining in prolapsed fascia inoculated with MSCs showed an increase in elastic fibers around these cells. This was not the case in prolapsed fascia inoculated with Vhcl.

II. RT-PCR analysis for COL I, COL III, MMP-2, TIMP-2, MMP-9, TIMP1, FBLN5 and ELN, performed in prolapsed fascia inoculated with MSCs respect to prolapsed fascia inoculated with Vhcl and normal fascia showed no statistical differences between the 3 groups for Col I. However, a clear trend towards normal fascia COL I levels was seen in prolapsed fascia inoculated with MSCs as compared with prolapsed fascia inoculated with Vhcl. COL III showed no differences between normal fascia inoculated with MSCs or Vhcl. Nevertheless, levels were lower than those observed in non prolpased fascia. COL I/ COL III ratio was higher in prolapsed fascia inoculated with MSCs than with Vhcl. This difference was statistically significant ($p<0.05$). Despite MMP-2, TIMP-2, MMP-9, TIMP1, FBLN5 and ELN didn't achieved significant differences between MSCs and Vhcl , their trends were towards the levels of non prolapsed tissues.

III. Immunohistofluorescence for Col I: In prolapsed fascia inoculated with MSCs, an intense signal in resident population cells around them was found, unlike prolapsed fascia inoculated with Vhcl. Normal fascia showed a strong Col I signal, according to the regular characteristics of this protein in healthy tissues. Image J assessment showed a statistically significant ($p < 0.05$) increase of Col I in prolapsed fascia inoculated with MSCs as compared with prolapsed fascia inoculated with Vhcl. Values observed in prolapsed fascia inoculated with MSCs were similar to those observed in non prolapsed fascia. FBLN5: Non prolapsed fascia showed a strong FBLN5 signal. No changes however in fluorescence intensity were observed in prolapsed fascia inoculated with MSCs versus Vhcl.

Interpretation of results

Our results show that MSCs may restore to ex vivo prolapsed tissues some of their original "pre prolapse" qualities. Col I / Col III relationship had statistical differences when measured with RT-PCR. Immunohistofluorescence showed statistical differences for Col I. The other markers tested did not reach statistical differences, but showed a clear trend towards it. Why is that? Probably the patients heterogeneity, either genetic or epigenetic, played an important role in this matter.

Concluding message

MSCs could play an important role in POP therapy, either as coadyuvant in primary prolapse surgery or as backup treatment in relapses. They could be administered in absorbable scaffolds, used as a vehicle that gives a transient structural support while MSCs repair the tissue (3). There's therefore the need to perform studies in animal models, in order to eventually do it in humans. It's a long road ahead.

To our knowledge, this is the first study that addresses the potencial rol of MSCs in prolapsed human tissues.

References

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Disclosures

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