

Evaluation of diabetes-associated testicular morphology and the effects of MSC secretome as a therapeutic intervention

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Background incl. aims

Diabetes is a metabolic disease characterized by prolonged hyperglycemia, which causes various types of complications, including impaired reproductive function. Diabetes-related microvascular damages, oxidative stress and insulin resistance could bring about a wide range of endocrine organ damages associated with the secretion of reproductive hormones and lead to hormonal imbalance, seminiferous tubule injury, including disrupted spermatogenesis and dysfunction of Sertoli cells. Ultimately, diabetes impacts male sexual function by leading to issues like erectile dysfunction, reduced libido and sperm damage. Mesenchymal stem cells (MSCs) could be a potential therapeutic intervention for the treatment of diabetes and accordingly, associated disorders like dysfunction of the male reproductive system¹. Conditioned media (CM) obtained from MSCs contain soluble and non-soluble factors, all of which are collectively considered as one of the best ways of conveying MSCs' therapeutic effects, including angiogenesis, anti-inflammatory and -apoptotic effects, immunomodulation, and promotion of tissue repair and regeneration. Preconditioning of MSCs with different strategies, like incubation in hypoxic or 3-dimensional (3D) cell culture conditions, could improve their therapeutic potential². In our previous study, the application of CM collected from MSCs cultured in 3D microfabricated scaffold to diabetic rats improved beta-cell regeneration and immunomodulation in comparison to the one obtained in conventional 2D culture conditions³. In this research, it was aimed to investigate the effects of prolonged hyperglycemia on serum reproductive hormone levels and testicular morphology of Sprague Dawley rats with diabetes, and the possible therapeutic effects of systemic application of CM derived from MSCs.

Methods

MSCs isolated from the human umbilical cord by tissue explant method were used for the collection of 2D-CM and 3D-CM. 22 rats were intraperitoneally treated with multiple low doses of streptozotocin (STZ; 5 days, 20 mg/kg) to induce diabetes. By the injection of last dose of STZ, it was confirmed that all the rats were in the range of diabetes (blood glucose level >250 mg/ml). Following the 2nd week of the first dose STZ injection, equal volumes (1 mL) of 2D-CM and 3D-CM were intraperitoneally applied to the diabetic rats (D+2D-CM, n=8; D+3D-CM, n=8) for 4 weeks as 3 doses a week. After 1 week of injection of the last dose, blood samples were collected by cardiac puncture for serum analysis of the hormones. The rats were sacrificed, and testis were obtained for light and transmission electron microscopic (TEM) evaluations. Serum concentrations of testosterone, gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were determined with enzyme-linked immunosorbent assay (ELISA). Light microscopic evaluation was performed with hematoxylin + eosin (H+E) staining. Semiquantitative Johnsen's tubular biopsy score (JTBS) analysis was used to histopathologically evaluate spermatogenesis out of an average 40 seminiferous tubules (STs). In this respect, STs were scored from 1 (no cell in the tubule section) to 10 (complete spermatogenesis and perfect tubules) in accordance with the level of epithelial maturation. Histomorphometric analyses were executed with the Fiji ImageJ software program by measuring the shortest diameter of ST and length of seminiferous epithelium (SE) (the distance between the basal lamina of SE and the closest spermatozoa to the lumen) out 10 STs for each specimen (n=3 for each group) at 40X

magnification. Statistical analyses were performed using SPSS version 20.0 software. $P < 0.05$ was accepted as statistically significant.

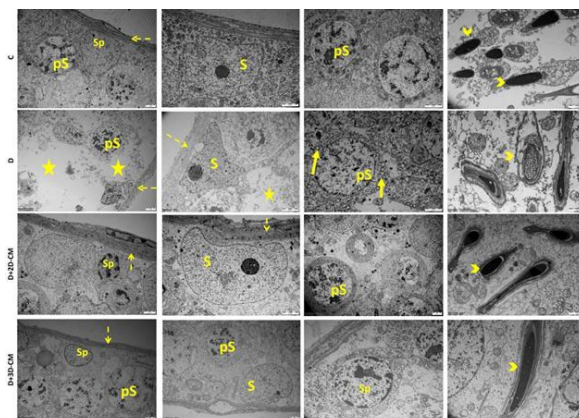
Results

ELISA analysis showed that serum LH and testosterone levels of experimental groups significantly decreased compared to the C group ($p < 0,05$). Serum GnRH and FSH levels were found to be significantly reduced in the D and D+2D-CM groups ($p < 0,01$ and $p < 0,05$ respectively), while there was no significant difference noted between the C and D+3D-CM groups. Our preliminary light microscopic analysis indicated that the JTBS values of experimental groups were significantly lower than the one of the C group while there was mild amelioration in D+3D-CM group in comparison to D group. On the other hand, our preliminary histomorphometric analysis of ST diameter and SE length did not show notable differences among the groups. TEM evaluation of SE revealed that the C group had normal ultrastructural morphology while spermatogonia with dispersed heterochromatin, primary spermatocytes having damaged mitochondria with loss of lamellar properties, cytoplasmic vacuolization in spermatogenic cell series and large intercellular spaces were noted in the D group. Additionally, assessments of spermatids at different stages of spermiogenesis demonstrated the presence of spermatids with disrupted chromatin condensation in the D group. In both treatment groups, such impairments were relatively fewer than the ones in the diabetic group and considerable restoration of ultrastructures of Sertoli and spermatogenic cells in seminiferous epithelium was observed.

Conclusion

In this diabetes model induced with multiple low doses of streptozotocin (5 days, 20 mg/kg), our preliminary light microscopic and TEM evaluations indicate that diabetes-associated damage in testis, specifically STs, was at a distinctive degree, and application of CM led to improvement in spermatogenesis and ultrastructural morphology of SE. On the other hand, when the differential changes in concentrations of serum reproductive hormones, including testosterone, GnRH, LH, and FSH, were considered, hyperglycemia seemed to affect the hypothalamic-pituitary-gonadal (HPG) axis by interrupting the feedback mechanism. The treatments with the CM, especially 3D-CM brought about considerable restoration of GnRH and FSH serum levels, but this was not enough to improve serum testosterone level, which is both a product and regulator of the HPG axis in males. The discrepancy between the morphological and hormonal analysis also suggests considering unrevealed mechanism of therapeutic action for MSCs derived CMs.

Graphic:



Graphic: Representative figures of TEM evaluations for C, D, D+2D-CM and D+3D-CM groups. S: Sertoli cells, Sp: spermatogonium, pS: primary spermatocyte, dashed arrow: basement membrane, arrowhead: spermatids at different stages of their development, star: intercellular space, arrow: mitochondria with loss of lamellar properties.

Keywords:

Mesenchymal-stem-cells, conditioned-medium, diabetes, testicular-damage, male-reproductive-hormones

Reference:

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