# The effect of mesenchymal stem cell's secretome on hyperglycemia-related complications: focus on reproductive system disorders

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Background incl. aims
Type 1 diabetes (T1D) is characterized by insulin deficiency due to the autoimmune destruction of β-cells in the pancreas, which causes hyperglycemia. Although exogenous insulin is very effective in increasing the survival of patients, inadequately controlled hyperglycemia leads to secondary complications. One of the complications associated with T1D is reproductive disorders. In males with long-standing T1D, this can appear as sexual dysfunction or reproductive issues, primarily stemming from impaired spermatogenesis, reduced testosterone production and release, and changes in glucose metabolism within Sertoli cells [1]. Mesenchymal stem cells (MSCs) hold promise for handling the therapeutic requirements of T1D due to their involvement in the immunomodulation process and cytoprotective, antiapoptotic, and antioxidant properties. It is established that MSCs exert their therapeutic effects via paracrine mechanisms, releasing a diverse array of bioactive molecules termed the secretome, which includes growth factors, cytokines, chemokines, exosomes, and other bioactive factors. The composition of the secretome can be modified through various inductions, presenting significant potential for targeted therapy development. Among these induction types are a hypoxic cell culture environment, cultivating the cells on a three-dimensional scaffold, and using various chemicals. Conditioned media (CM) represents a tool to use the therapeutic benefits of the secretome exclusively, obtained by collecting the medium in which MSCs are cultured, free of cellular components [2]. The CM is important in the treatment of T1D due to the factors it contains [3]. Furthermore, the systemic application of CM could be crucial in preventing and repairing tissue damage caused by hyperglycemia in the body, despite this not being the primary treatment goal. This comprehensive approach is valuable as it addresses the entirety of T1D, which affects the entire organism. So, this study aims to investigate the effects of human umbilical cord MSC-derived CM, used for therapeutic purposes in the rat experimental T1D model, on testicular damage and the disrupted hormone mechanism, caused by hyperglycemia.
Methods

MSCs were isolated from human umbilical cord tissue by the tissue explant method, followed by characterization experiments. Two distinct CM types were obtained: CM obtained from MSCs cultured in normal culture (N-CM) conditions and CM obtained from MSCs preconditioned with a hypoxia mimetic agent, deferoxamine (150 µM) (DFX-CM). Sprague-Dawley rats were used for in vivo experiments, and the T1D model was induced using a single high-dose of streptozotocin (55 mg/kg). Experimental groups included control (C), diabetes (D), diabetes with N-CM treatment (D+N-CM), and diabetes with DFX-CM treatment (D+DFX-CM), with 6 rats in each group. Following the 4th week of the STZ injection, diabetic rats received intraperitoneal injections of equal amounts of N-CM and DFX-CM (each containing at least 15 µg of protein) four times a week for three weeks. Blood samples for hormone analysis were collected via cardiac puncture one week after the final CM dose injection, and the rats were sacrificed. After sacrification, serum levels of gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were quantified via enzyme-linked immunosorbent assay (ELISA) for hormonal profiling. Testicular tissues stained with hematoxylin and eosin were used to perform Johnsen's scoring via light microscopy to assess spermatogenesis. For this analysis, an average of 10 seminiferous tubule

sections were analyzed in each rat (n=3). Seminiferous tubule diameter and epithelial thickness were also measured using the Image J analysis program. Finally, cellular ultrastructure was examined using transmission electron microscopy (TEM). All findings are presented as preliminary outcomes. Statistical analysis was conducted using the Kruskal-Wallis test, with p-values <0.05 considered significant.

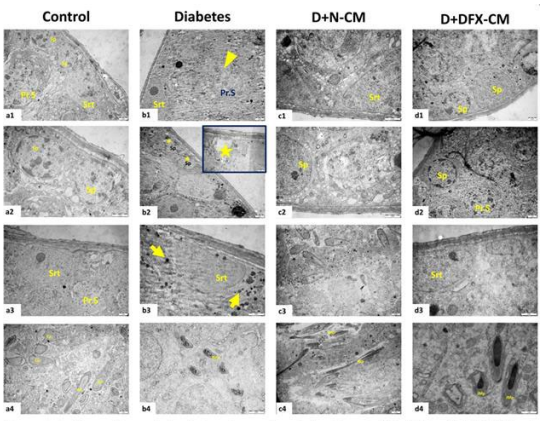
Results

Upon examination of reproductive hormone levels in serum, statistical significance was identified among the groups for GnRH, FSH, and LH ($p<0.05$, $p<0.001$, and $p<0.05$, respectively). All hormone levels decreased in the D group. It was noteworthy that GnRH, FSH, and testosterone levels tended to increase in the D-DFX-CM group compared to the D group. Johnsen's scoring indicated impairment in spermatogenesis in the diabetes groups. On the other hand, the measurements of seminiferous tubule diameter and epithelial thickness did not reveal statistically significant differences. TEM analysis of seminiferous tubules revealed that the C group exhibited normal morphology. In contrast, the D group exhibited elevated lysosome levels in Sertoli cells, spermatogonia with dispersed heterochromatin, and primary spermatocytes with disintegrated nuclear membranes, alongside increased intercellular spaces. In both treatment groups, such defects were relatively less than in the diabetic group, and a substantial improvement in the ultrastructure of Sertoli and spermatogenic cells within the seminiferous epithelium was observed. Examination of spermatids showed that some in the diabetes group had impaired chromatin condensation. Axoneme structures were generally preserved (Fig.1).

Conclusion

Ultrastructural examinations revealed that the secretome of MSCs improved the seminiferous tubule epithelium by exerting a cytoprotective effect. Despite no significant impact on the hypothalamic-pituitary-gonadal axis, which is impaired by hyperglycemia, it is noteworthy that DFX-CM increased GnRH and testosterone release from the hypothalamus. However, this effect was insufficient, leading to no observable improvement in spermatogenesis. These preliminary findings underscore the necessity for further investigation into the observed ultrastructural improvement in the seminiferous epithelium and the need to uncover new cellular-level data elucidating the mechanisms of action of the secretome of MSCs.

**Graphic:**




Representative figures of transmission electron microscopic evaluations for control (**a**), diabetes (**b**), D+N-CM (**c**) and D+DFX-CM (**d**) groups. **Sp**: Spermatogonium, **Pr.S**: Primary spermatocytes, **Srt**: Sertoli cells, **Cp**: Cap phase, **Ap**: Acrosomal phase, **iMp**: initial maturation phase, **Mp**: maturation phase, **arrow**: lysosome, **star**: intercellular space, **asterisk**: dispersed heterochromatin of Sp and **arrow head**: disintegrated nuclear membrane

**Keywords:**

Mesenchymal stem cells-Testicular damage-Conditioned medium

**Reference:**

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