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Intracavernous stem cell transplantation in erectile dysfunction: modification of adhesive properties of stem cells increases their ability to restore contractile and phosphatase activity of cavernous tissue damaged by hyperglycemia

I. Gorpynchenko, A. Sytenko
Institute of Urology of the National Academy of Medical Sciences of Ukraine

A comparative study of the effect on the contractile and phosphate activity of the cavernous tissue of four intracavernous injections (ICI) (1×10^6 cells once a week) of native stem cells (SC) and cells, with SYT1 / SYT2 molecules, integrated into the cell membrane (SC-m-SYT1 / SYT2), was performed in two groups (n=10; 1:1) of sexually mature Wistar rats weighing 160-180 g with induced hyperglycemia (>8 mmol/l). It was found that, at the end of the ICI in the group of animals receiving SC-m-SYT1 / SYT2, the median amplitude of contractions was 1.6 times greater (P<0.05, Wilcoxon's test), and the intensity mediants for ATPase and LF, respectively, 2 and 1 points greater than in the group where native SCs were introduced (P<0.05, Wilcoxon's test). ICI of native and modified SC did not affect the level of glycemia.

Modification of the adhesive properties of SC by SYT1 / SYT2 molecules increases their ability to restore contractile and phosphatase activity of cavernous tissue damaged by hyperglycemia. Further research is needed on the processes of homing and differentiation of SC, modified with SYT1 / SYT2 molecules, in intact and pathologically altered cavernous tissue in vitro and in vivo.

Key words: *erectile dysfunction, stem cells, hyperglycemia, intracavernous transplantation, cavernous tissue, contractile activity, phosphatase activity.*

The development of new highly effective methods of treating erectile dysfunction (ED) is an urgent task of urological science. Despite the fact that the development of ED is associated with age, this disease affects a significant number of men of sexually active age (up to 40 years) [1]. Moreover, this pathology has a significant negative effect on the psycho-emotional state of the patient and his sexual partner, leads to the development of dysrhythmias, reduces self-esteem, violates the harmony of interpersonal relationships and, thus, worsens the quality of life [2, 3]. The situation is complicated by the fact that a very high percentage of patients (almost 50%) refuses to use phosphodiesterase type 5 inhibitors – the first-line drugs for the treatment of erectile dysfunction, after a certain period of time [4, 5]. In addition, this group of drugs does not restore the structure and function of the cavernous tissue, but only potentiates the erection. On the other hand, phalloprosthetics has not become popular due to traumatic and physiological.

A promising direction of solving these problems may be the use of stem cells (SC), in order to restore the structure and functional properties of cavernous tissue. This is evidenced by the first experience of their intracavernous transplantation. However, as it turned out, with a significant improvement in the structural and functional parameters of the cavernous tissue, a very small amount of SC can be identified in it itself [6–16]. This may be due to migration or death of cells, which, on the one hand, reduces their therapeutic effect, and on the other hand, it worsens the selectivity of the action, since SC with blood flow can fall into different parts of the body with unpredictable consequences.

To solve the problem of the low adhesion of the SC to the subendothelial matrix, we proposed to integrate into their membrane special molecules (SYT1 and SYT2). In the previous experiment, we demonstrated that the expression of SC in media SYT1 and SYT2 leads to the inclusion of these molecules in the cellular membrane. Thus, the proportion of SYT1 and SYT2 positive SC in pools exposed in special SYT1 / SYT2 environments was significantly ($p<0.05$) higher, respectively, by 70% and 59%, and 64% and 48%, respectively, than in exposed pools Sham and SYT1 / SYT1 solutions.

In this work, we examined the assumption that SC with modified adhesive properties (SC-m-SYT1 / SYT2), more clearly than the native IC, to restore the phosphatase and contractile activity of the cavernous tissue damaged by hyperglycemia.

MATERIAL AND METHODS

According to the experiment, two intravenous injection (ICI) ICs (1×10^6 cells) were administered once weekly to group A for two groups of adult male rats in the Vistar line from streptozotocin induced by hyperglycemia (glucose level >8 mmol/l) for 3 months. (n=10) were administered SC-m-SYT1 / SYT2, group B (n=10) native SK. One week after the last ICH in animals, blood glucose levels from the caudal vein were determined and deduced from the experiment. The penis was removed, after which it was isolated from the pelvic tissue for further determination of phosphatase (alkaline phosphatase (LF) and ATPase) and contractile activity.

Experimental studies were performed on 20 sexually mature male Wistar rats weighing 160–180 g, vivarium dilution of the Institute of Pharmacology and Toxicology of the National Academy of Medical Sciences of Ukraine, which were on the standard diet in accordance with sanitary and hygiene norms, the rules of the European Convention for the Protection of Vertebrates animals used for experimental or other purposes (Strasbourg, 1986). The initial glucose level in all animals was in the range of 4.0–5.11 mmol/l.

Methods of induction of hyperglycemia, glucose test, determination of phosphatase activity of cavernous tissue, and characteristic of native SC are described in previous publications [17, 18]. The isometric force of reducing cavernous tissue strips in response to an electrical impulse (voltage 50 V, duration 10 s, frequency range (1–32 Hz)) was determined according to the standard method.

Characteristics of the studied groups *

Parameter	Group A, n=10	Group B, n=10	p
Body weight, g	161 (147; 167)	157 (149; 167)	>0,05
The level of glycemia, nmol/l	36 (28; 38)	35 (30; 38)	>0,05
Amplitude of contractions	3,6 (3,1; 3,9)	2,3 (2,1; 2,7)	<0,05
Intensity of staining on ATP-aze, points	4 (4; 5)	3 (3; 4)	<0,05
Intensity of painting on LF, points	5 (4; 5)	3 (3; 4)	<0,05

* Data transmitted as Me (Persentil 25; 75).

For the modification of adhesive properties, native SCs were exposed in a special medium containing SYT1 / SYT2 molecules.

Statistical analysis: The reliability of intergroup differences in median values of the amplitude of contractions and the intensity of coloration was determined by the Wilcoxon criterion. The level of significance was assumed to be 0.05.

RESULTS

Prior to ICI, both groups of animals were comparable in terms of age and level of glycemia (Table 1).

During experiments with electrostimulation, it was found that strips of cavernous tissue obtained from the male hippoglycemic male rats of the Wistar line, which were introduced SC-m-SYT1 / SYT2, reacted to the action of electric current with reductions of greater amplitude than strips of animals that were introduced by native MSCs (Figure 1).

The Wilcoxon test confirmed that the differences between the groups at the median values of the amplitude of the reduction (Table) were non-random.

In the previous work [17] we found that for intact cavernous tissue there is a high activity of AF and ATP-bases, which is predominantly determined in endothelial cells (the endothelial lining of sinuses and capillaries is completely painted in a dark brown color). Hyperglycemia reduces the activity of ATPase and AF, which is determined by the unevenness of the dye deposition and the reduction of the intensity of coloration. At the end of the course of the ICI in the group of animals transplanted MSC-m-SYT1/ SYT2, a significantly more restful activity of both enzymes was recorded, compared with the group that introduced native cells.

In particular, Me intensity of coloring on AF and ATPase in group A were significantly ($p<0.05$) were respectively 1.5 and 1.6 times higher than in group B.

Analysis of glycemic changes in both groups before and after transplantation did not reveal statistically significant effects from both modified and native SCs on this indicator.

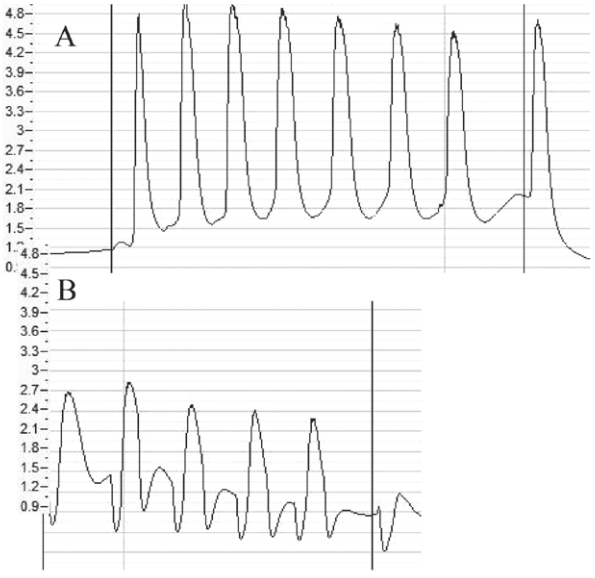

Fig. 1. Curves of strips of cavernous tissue in response to an electrical impulse: A - from the hyperglycemic male rat # 3A, received four IICs MSC-m-SYT1 / SYT2 (large amplitude of abbreviations); B – from a hyperglycemic male rat, number 5B, which received four IICs of native MSCs (small amplitude of contractions)

Thus, the recorded effects – a more pronounced restoration of the contractile ability and phosphatase activity of the cavernous coccidia in the group using SC-m-SYT1 / SYT2, in comparison with the group where the native ICs were used – indicate that inclusion in the membrane of stem cells of SYT1 molecules

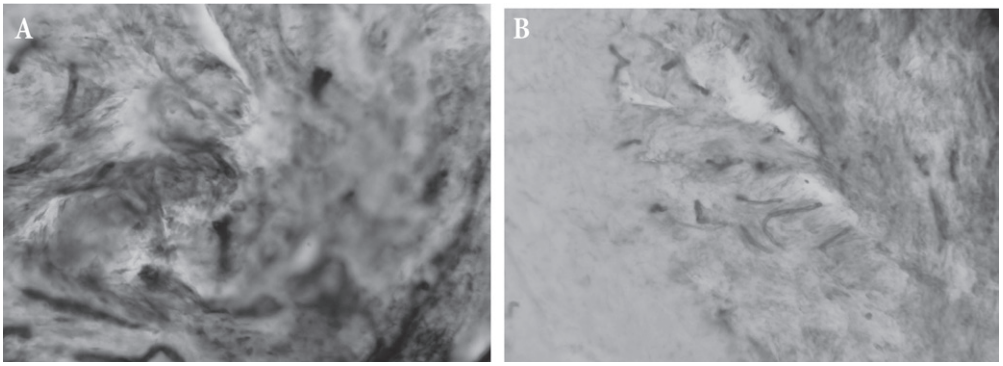

Fig. 2 The activity of ATPase in the rat's caudal tissue: A – from the hyperglycemic male rat 3A, which received four IICs SC-m-SYT1 / SYT2 (intense and uniform accumulation of dye in the endothelial lining area); B – from a hyperglycemic male rat, number 5B, which received four IVIs of native SC (low-intensity focal deposition of the dye). Vachstein and Meisel methods. Magnification ×200

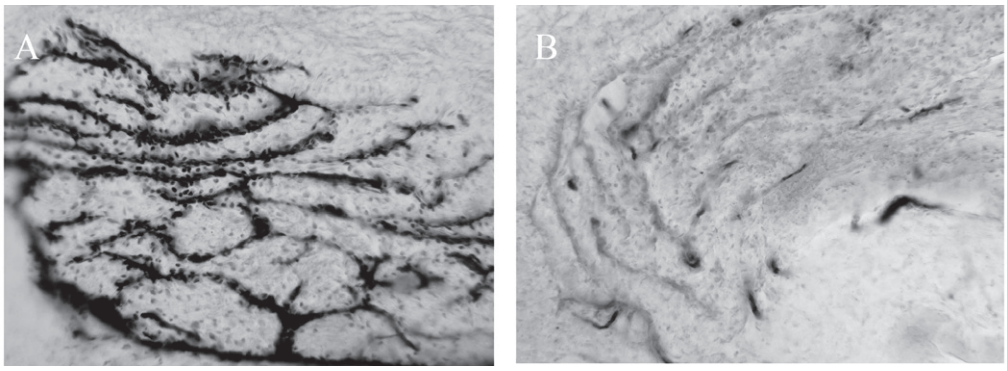

Fig. 3. AF activity in rats' cavernous tissue: A – from hyperglycemic male rats # 3A receiving four IICs SCMSYT1 / SYT2 (intense and uniform accumulation of dye in the endothelial lining area); B – from a hyperglycemic male rat, number 5B, which received four IVIs of native SC (low-intensity focal deposition of the dye). Methodology of Gomori. Magnification ×200

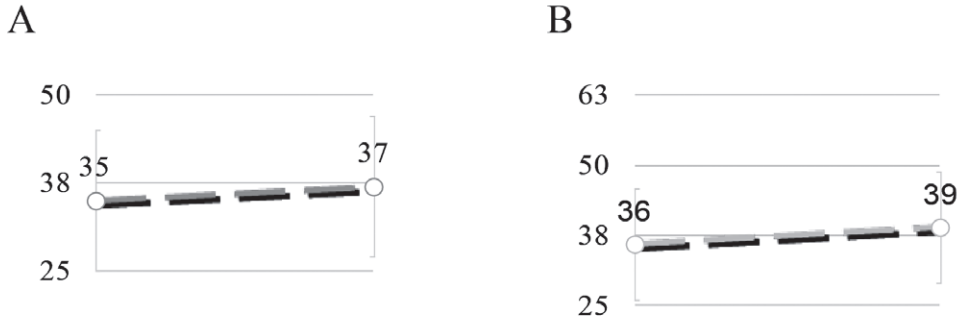

Fig. 4. Dynamics of hyperglycemia in the studied groups

and SYT2 using special vectors increases their ability to be fixed to the subendothelial matrix. Further research is needed on the processes of differentiation of modified SC in intact and pathologically modified pelvic tissue of the penis.

CONCLUSIONS

1. Modification of the adhesive properties of SC with the help of SYT1 / SYT2 molecules increases their ability to restore the crescent activity of the cavernous tissue of male rats in the Vistar line damaged by hyperglycemia: the median amlitum of the contractions of the smooth muscle strips from animals contracted by SC-m-SYT1 / SYT2 was in 1.6 times greater ($p<0.05$, Wilcoxon's criterion) than in the group where native ICs were used.

2. Modification of adhesive properties of SC with the help of molecules SYT1 / SYT2, enhances their ability to restore the phosphatase activity of the cavernous tissue of male rats in the Vistar line damaged by hyperglycemia: the median intensity of coloring by ATP-aza and LF of pathogistological preparations of cavernous tobaccos from animals that have been grafted SC-m – SYT1 / SYT2, was 2 and 1 points higher ($p<0.05$, Wilcoxon criterion) than in the group where native ICs were used.

3. Intracavernous transplantation of both modified and native SCs does not lead to correction of the level of glycemia in the experimental animals, and therefore the influence of SC on the cavernous tissue is associated with their paracrine effects.

Further research on humming processes and differentiation of SC modified by SYT1 / SYT2 molecules in intact and pathologically modified cavernous coccidia in vitro and in vivo are necessary.

Интракавернознозная трансплантация при эректильной дисфункции: модификация адгезивных свойств молекулами SYT1/SYT2 усиливает способность стволовых клеток восстанавливать сократительную и фосфатазную активность пещеристой ткани, поврежденной в результате гипергликемии

И.И. Горпинченко, А.М. Сытенко

На двух группах (n=10; 1:1) половозрелых крыс-самцов линии Вистар массой 160–180 г с индуцированной гипергликемией (>8 ммоль/л) проведено сравнительное исследование влияния четырех интракавернозных инъекций (1×10^6 клеток один раз в неделю) нативных стволовых клеток (СК) и клеток с интегрированными в мембрану молекулами SYT1 / SYT2 (СК-м-SYT1 / SYT2) на сократительную и фосфатазную активность пещеристой ткани. Установлено, что по окончании ИКИ в группе животных, получавших СК-м-SYT1 /SYT2, медиана амплитуды сокращений была в 1,6 раза больше ($p<0,05$, критерий Вилкоксона), а медианы интенсивности окраски на АТФ-азу и ЛФ соответственно на 2 и 1 баллов больше, чем в группе, где вводились нативные СК ($p<0,05$, критерий Вилкоксона). ИКИ нативных и модифицированных СК не влияли на уровень гликемии.

Модификация адгезивных свойств СК молекулами SYT1 / SYT2 повышает их способность восстанавливать сократительную и фосфатазную активность пещеристой ткани, поврежденной вследствие гипергликемии. Необходимы дальнейшие исследования процессов хоуминга и дифференцировки СК, модифицированных молекулами SYT1 / SYT2, в интактной и патологически измененной пещеристой ткани in vitro и in vivo.

***Ключевые слова**: эректильная дисфункция, стволовые клетки, гипергликемия, интракавернозная трансплантация, пещеристая ткань, сократительная активность, фосфатазная активность.*

Інтракавернозна трансплантація при еректильній дисфункції: модифікація адгезивних властивостей молекулами SYT1 / SYT2 посилює здатність стовбурових клітин відновлювати скоротливу та фосфатазну активність печеристої тканини, ушкодженої внаслідок гіперглікемії

І.І. Горпинченко, А.М. Ситенко

На двох групах (n=10; 1:1) статевозрілих щурів-самців лінії Вістар масою 160–180 г з індукованої гіперглікемією (>8 ммоль/л) проведено порівняльне дослідження впливу чотирьох інтракавернозних ін'єкцій (1×10^6 клітин один раз на тиждень) нативних стовбурових клітин (СК) і клітин з інтегрованими у мембрану молекулами SYT1 / SYT2 (СК-м-SYT1 / SYT2), на скоротливу і фосфатазну активність печеристої тканини. Встановлено, що по закінченню ІКД у групі тварин, які отримували СК-м-SYT1 / SYT2, медіана амплітуди скорочень була в 1,6 разу більше ($p<0,05$, критерій Вілкоксона), а медіани інтенсивності забарвлення на АТФ-азу і ЛФ відповідно на 2 і 1 балів більше, ніж в групі, де вводилися нативні СК ($p<0,05$, критерій Вілкоксона). ІКД нативних і модифікованих СК не впливали на рівень глікемії.

Модифікація адгезивних властивостей СК молекулами SYT1 / SYT2 підвищує їхню здатність відновлювати скоротливу і фосфатазну активність печеристої тканини, пошкодженої внаслідок гіперглікемії. Необхідні подальші дослідження процесів хоумінга і диференціювання СК, модифікованих молекулами SYT1 / SYT2, в інтактній і патологічно зміненій печеристій тканині in vitro і in vivo.

Ключові слова: *еректильна дисфункція, стовбурові клітини, гіперглікемія, інтракавернозна трансплантація, печериста тканина, скоротлива активність, фосфатазна активність.*

Сведения об авторах

Горпиченко Игорь Иванович – ГУ «Институт урологии НАМН Украины», 04053, г. Киев, ул. В. Винниченко, 9а

Сытенко Андрей Михайлович – ГУ «Институт урологии НАМН Украины», 04053, г. Киев, ул. В. Винниченко, 9а. *E-mail: andrew.sytenko@gmail.com*

REFERENCES

1. Capogrosso P., Colicchia M, Ventimiglia E, Castagna G, Clementi MC, Suardi N, Castiglione F, Briganti A, Cantiello F, Damiano R, Montorsi F, Salonia A. One patient out of four with newly diagnosed erectile dysfunction is a young man-worrisome picture from the everyday clinical practice. //J Sex Med. 2013 Jul;10(7):1833-41.

2. Emanu J.C., Avildsen I.K., Nelson C.J. Erectile dysfunction after radical prostatectomy: prevalence, medical treatments, and psychosocial Interventions//Curr Opin Support Palliat Care. 2016 Mar; 10(1): 102–107.

3. Mourikis I., Antoniou M., Matsouka E., Vousoura E., Tzavara C., Chrysa Ekizoglou, Papadimitriou G.N., Vaidakis N., Zervas I.M. Anxiety and depression among Greek men with primary erectile dysfunction and premature ejaculation// Ann Gen Psychiatry. 2015; 14: 34.

4. Hatzimouratidis K., Hatzichristou D. Phosphodiesterase type 5 inhibitors: unmet needs. // Curr. Pharm. Des. 2009; 15(30): 3476-85.

5. Jiann B.P., Yu C.C., Su C.C., Tsai J.Y. Compliance of sildenafil treatment for erectile dysfunction and factors affecting it.// Int J Impot Res. 2006 Mar-Apr;18(2):146-9.

6. Kwon E.B., Lee J.Y., Piao S., Kim I.G., Ra J.C., Lee J.Y. Comparison of human muscle-derived stem cells and human adipose-derived stem cells in neurogenic trans-differentiation. – Korean J Urol. 2011. – 52 (12). – 852–857.

7. Lin G., Banie L., Ning H., Bella A.J., Lin C.S., Lue T.F. Potential of adipose-derived stem cells for treatment of erectile dysfunction// J. Sex Med. – 2009. – 6 Suppl 3:320-327.

8. Ning H., Liu G., Lin G., Yang R., Lue T.F., Lin C.S. Fibroblast growth factor 2 promotes endothelial differentiation of adipose tissue-derived stem cells// J. Sex. Med. – 2009. – 6 (4). – P. 967–979.

9. Gholami S.S., Rogers R., Chang J., Ho H.C., Grazziottin T., Lin C.S., Lue T.F. The effect of vascular endothelial growth factor and adeno-associated virus mediated brain derived neurotrophic factor on neurogenic and vasculogenic erectile dysfunction induced by hyperlipidemia// J. Urol. – 200. – 3;169. – P. 1577–1581.

10. Hristov M., Weber C. Endothelial progenitor cells: characterization, pathophysiology, and possible clinical relevance.// J Cell Mol Med 2004;8:498–508.

11. Vasa M., Fichtlscherer S., Aicher A., Adler K., Urbich C., Martin H., Zeiher A.M., Dimmeler S. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease.// Circ Res 2001;89:E1–7.

12. Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, Epstein SE. Marrow-derived stroll cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms// Circ Res 2004;94:678–685.

13. Dai W, Hale SL, Martin BJ, Kuang JQ, Dow JS, Wold LE, Kloner RA. Allogeneic mesenchymal stem cell transplantation in postinfarcted rat myocardium: short- and long-term effects. Circulation 2005;112:214–223.

14. Oswald J, Boxberger S, Jorgensen B, Feldmann S, Ehninger G, Bornhauser M, Werner C. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. Stem Cells 2004;22:377–384.

15. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002;13:4279–4295.

16. De Ugarte DA, Morizono K, Elbarbary A, Alfonso Z, Zuk PA, Zhu M, Dragoo JL, Ashjian P, Thomas//J Am Coll Cardiol. – 2005. – 45:982–988.

17. Горпинченко І.І., Ситенко А.М., Бухтіарова Т.А., Ядловський О.Є., Матвієнко А.В., Попандопуло А.Г., Кавеліна А.С. Вплив інтракавернозних ін'єкцій кістковомозкових мезенхімальних стовбурових клітин на морфологію та фосфатазну активність печеристої тканини щурів-самців із стретозотоциніндукованим цукровим діабетом // Здоровье мужчины, № 4 (47), 2013. – С. 142–145.

18. Пат. № 98906 UA, МПК C12Q 1/42, G01N 33/50 (2006.01). Спосіб оцінки стану ендотелію печеристої тканини статевого члена /Горпинченко І.І., Ситенко А.М., Ядловський О.Є., Матвієнко А.В. (UA); ДУ «ІУНАМНУ», ДУ «ІФТНАМНУ» (UA); № u201412923, 03.12.2014; Опуб. 12.05.2015, Бюл. № 9.

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