Cavernous nerve reconstitution with the use of bone marrow stem cells and erectile function evaluation: an experimental animal study

Avaliação da função erétil após a reconstituição do nervo cavernoso com o uso de células-tronco de medula-óssea: estudo experimental em ratos

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ABSTRACT

Objective: To assess the influence of adult stem cells from bone marrow of rats in the regeneration of cavernous nerve, taking the return of erectile function as a parameter in animals subjected to the apomorphine-induced test of erection. Methods: Forty-eight male Wistar-EPM rats, aged between nine and ten weeks, and weighing approximately 250 g were used. They were randomly divided into four study Groups containing 12 animals each, as follows: Group I: surgical exposure of the cavernous nerves bilaterally without injury; Group II: bilateral surgical injury of the cavernous nerve of approximately 3 mm, without reconstruction; Group III: bilateral surgical injury of the cavernous nerves of approximately 3 mm, and bilateral reconstruction with silicone guiding tubes containing saline solution inside; Group IV: bilateral surgical injury of the cavernous nerves of approximately 3 mm, and bilateral reconstruction with silicone guiding tubes filled with adult stem cells. Four weeks after surgery, the animals were injected with apomorphine for induction of erection. Results: In Group I there was complete erectile response in all animals (100% – 12 out of 12). On the other hand, none of the animals in Group II presented erection after the use of apomorphine. Five of the twelve animals of Group III (41.7%) and nine of the 12 animals of Group IV (75%) had erections after the stimulus. When we compared the frequency of restoration of erection in the four Groups, Group IV was shown to have a similar performance to Group I (p = 0.217), while Group III animals had a frequency of erections inferior to those in Group I (p = 0.005). Moreover, comparison of results of Groups III and IV versus Group II showed that the frequency of erections was statistically higher in the first two Groups (p = 0.037 and p < 0.001, respectively). Finally, Group IV presented a tendency to a larger number of erections when compared to Group III (75% versus 41.7%) but this difference was not statistically significant (p = 0.098). Conclusion: This study shows that adult stem cells from bone marrow, filling silicone guiding tubes, may promote the regeneration of cavernous nerves and restore erectile function in an animal model.

Keywords: Adult stem cells; Bone marrow cells; Penile erection/drug effects; Apomorphine; Rats, Wistar; Epidemiology, experimental; Peripheral nerves

RESUMO

Objetivo: Avaliar a influência de células-tronco adultas da medula óssea de ratos na regeneração do nervo cavernosos lesado, tomando-se como parâmetro o retorno da função erétil nos animais submetidos ao teste de ereção induzido pela apomorfina. Métodos: Quarenta e oito ratos Wistar-EPM machos, com idades entre nove e dez semanas, pesando aproximadamente 250 g, foram usados e randomicamente subdivididos em quatro grupos de estudo contendo 12 animais cada. Os grupos experimentais foram divididos em: Grupo I: exposição cirúrgica bilateral do nervo cavernoso sem lesão; Grupo II: lesão cirúrgica bilateral do nervo cavernoso. Grupo III: lesão cirúrgica de controle sem reconstrução. Grupo IV: lesão cirúrgica bilateral do nervo cavernoso e reconstrução com tubos de silicone. Após quatro semanas de cirurgia, os animais foram injetados com apomorfina para indução de ereção. Resultados: No Grupo I houve resposta completa de ereção em todos os animais (100% – 12 out of 12). Já no Grupo II, nenhuma das oito animais apresentou ereção após o uso de apomorfina. Dezenove por cento (20%) dos animais do Grupo III (61%) e 75% dos animais do Grupo IV tiveram ereções após o estímulo. Quando analisados os grupos, o Grupo IV apresentou frequência de ereções similar ao Grupo I (p = 0.217), enquanto os animais do Grupo III apresentaram frequência de ereções inferior à do Grupo I (p = 0.005). A comparação entre os grupos III e IV com o Grupo II mostrou que a frequência de ereções foi estatisticamente superior nos primeiros dois grupos (p = 0.037 e p < 0.001, respectivamente). Finalmente, o Grupo IV apresentou tendência a um maior número de ereções quando comparado ao Grupo III (75% versus 41.7%) mas esta diferença não foi estatisticamente significante (p = 0.098). Conclusão: Este estudo mostra que células-tronco adultas da medula óssea, preenchendo tubos de silicone, podem promover a regeneração de nervos cavernosos e restaurar a função erétil em um modelo animal.

Keywords: Adult stem cells; Bone marrow cells; Penile erection/drug effects; Apomorphine; Rats, Wistar; Epidemiology, experimental; Peripheral nerves

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bilateral of the cavernous nerves of approximately 3 mm, and reconstruction bilateral with guide-wires of silicone containing solution saline in its interior. Group IV: surgical lesion bilateral of the cavernous nerves of approximately 3 mm, and reconstruction bilateral with guide-wires of silicone sown with adult stem cells of bone marrow in its interior. Four weeks after surgery, the animals were subjected to apomorphine to induce erection. Results: No group I observed complete erectile response in all animals (100% – 12 of 12). By the other hand, none of the animals of group II presented erections after administration of apomorphine. Five of the doze animals of the Group III (41.1%) presented erections, whereas no erections were observed in the Group IV (75%).

INTRODUCTION

Prostate cancer is the most common non-skin cancer in men, with approximately 186,300 new cases expected for 2008 in the United States. Nowadays, radical retropubic prostatectomy, which is indicated for the treatment of localized prostate cancer, is accepted as a form of curative treatment. However, this technique has been responsible for many of the cases of erectile dysfunction. Tension, compression, and laceration are the major mechanisms of peripheral nerve injury during radical prostatectomy, leading to degeneration of these nerves. The neuronal regeneration begins close to the proximal stump just a few hours after injury, but factors such as time and extent of the defect created are fundamental to an adequate reintegration.

The development of new techniques like the neurovascular bundle preservation, intraoperative electrostimulation and the use of neural autologous transplants to restore the communication of cavernous nerves has minimized the degree of neuronal damage. However, it is necessary to create new methods of restoring the cavernous nerves since the current indications for the use of autologous neural grafts are still uncertain.

Reinnervation after resection and cavernous nerve grafting was demonstrated for the first time in an animal model by Quinlan (1989) and Burgers (1988). Ball et al. described a technique for reconstitution of the cavernous nerve using silicone tubes filled with Matrigel®, Becton, Dickinson and Company, FranklinLagos, New Jersey, United States) or fibroblastic growth factors in an animal model with positive results (10-11).

May et al. (2002) presented a major step ahead toward the use of cavernous nerve reconstitution. They suggest that artificial conduits, with or without the help of Schwann cells, may be more reliable than their biological counterparts, such as the sural nerve, in regeneration.

Primordial cells from bone marrow contribute to the regeneration and revascularization of ischemic tissue in various diseases. It has been shown that the bone marrow is a rich source of stem and progenitor cells that can mobilize quickly to the ischemic sites and infiltrate the parenchyma, which give rise primarily to microglia or endothelial cells and to a small number of cells that express neuronal markers and astrocytes.

It is well established in the literature that the hematopoietic system is an excellent source of cells for transplant and manipulation. Bone marrow cells are more accessible and easier to manipulate than neural stem cells. The recent identification of two categories of adult stem cells in bone marrow, mesenchymal and hematopoietic stem cells with different capabilities of differentiation, confirmed the importance of this structure in cell therapy.

This study investigated the influence of adult stem cells from bone marrow of rats in the regeneration of cavernous nerve, taking the return of erectile function as a parameter in animals subjected to the apomorphine-induced test of erection.

OBJECTIVE

To assess the influence of adult stem cells from bone marrow of rats in the regeneration of cavernous nerve, taking the return of erectile function as a parameter in animals subjected to the apomorphine-induced test of erection.

METHODS

Forty-eight Wistar-EPM male rats, aged between nine and ten weeks, weighing approximately 250 g, were used and randomly divided into four groups of study containing 12 animals each. The experimental groups were divided into:
- Group I: surgical exposure of the cavernous nerves bilaterally without injury;
- Group II: bilateral surgical injury of the cavernous nerves of approximately 3 mm, without reconstruction;
- Group III: bilateral surgical injury of the cavernous nerves of approximately 3 mm, and bilateral reconstruction with silicone guiding tubes containing saline solution inside;
- Group IV: bilateral surgical injury of the cavernous nerves of approximately 3 mm, and bilateral reconstruction with silicone guiding tubes containing adult stem cells.

Four weeks after surgery, the animals were injected with apomorphine for induction of erection.

The surgery was done under ketamine and xylazine anaesthesia. Isothermia was maintained at 37 °C by placing the rats on a heating pad. A lower abdominal midline incision was made from the symphysis pubis to the mid-abdomen. The testes were retracted, their gubernacula were divided and they were packed into the upper abdomen. An operating microscope aided the dissection. The cavernous nerve exited the major pelvic ganglion in the groove between the urethra and rectum, dividing under the symphysis and finally innervating the bulbous urethra and corpora cavernosa (Figures 1-3). There were also numerous fine nerve fibers coursing from the major pelvic ganglion in all directions toward the pelvic viscera. The endopelvic fascia overlying the cavernous nerve was incised and, after periprostatic dissection, the nerve and the major pelvic ganglion were identified posterolaterally on either side of the prostate. Then the nerve was sectioned and resected on both sides. After nerve resection in groups III and IV, cavernous nerve reconstitution was performed with silicone tube containing saline solution or adult stem cells inside.

Bone marrow samples were obtained from adult Wistar-EPM rats (250 g) femurs. The epiphyses were removed, and the medullary cavity was washed with DMEM (Dulbecco’s Modified Eagle Medium – GibcoBRL) without serum. The material was centrifuged at 1,500 rpm, for 10 minutes. The pellet was resuspended in DMEM 4 ml without serum and transferred to another tube containing Ficoll-Paque™ (density 1.077 g/mL – Amersham Biosciences). Then 2,000 rpm for 30 minutes was used for a new centrifugation process. After that, the ring of cells was separated from Ficoll-Paque™, and resuspended in a BSS solution with a 1:5 dilution (cells: BSS). Three more times all the material was centrifuged at 1,500 rpm for 10 minutes each time. All Ficoll-Paque™ was thus removed, due to its cell toxicity.

At the end, the pellet was resuspended in DMEM 1 ml containing 20% of fetal bovine serum (SFB – StemCell Technology). The cells were then counted and accessed for viability using Trypan Blue (Gibco-BRL). Finally, the cells were plated in Corning flasks with canted neck filter, 25 cm², with 5 mL of culture medium composed of DMEM 20% SFB plus 1% penicillin and streptomycin (StemCell Technology) and 4% of L-glutamine (GibcoBRL); 10⁶ cells were plated and kept in oven at 37 °C in 5% CO₂ and humidity of 95%. The culture medium was changed every three or four days, taking out 4 ml of conditioned medium and replacing them by 4 ml of fresh medium.

When the cells reached confluence in culture for approximately 80%, the change was made. Removing the culture medium from the bottle, the mesenchymal cells remained attached to the bottle. These cells were washed with DMEM without serum to remove...
residual SFB and then incubated with 1 ml of trypsin and EDTA (StemCell Technology), at 37 °C, until the stress (approximately four minutes). Culture medium with 20% of SFB was added to the already loose cells to neutralize trypsin. The cells were centrifuged at 1,200 rpm for five minutes, then the supernatant was removed and the pellet resuspended in culture medium (DMEM + 20% SFB). These cells were further divided into 25 cm² flasks for culture. The culture of cells underwent four passages until the completion of surgery.

During surgery all animals from group IV received 250,000 cells/200 µl on each side of the anastomotic site. Four weeks after the procedure, the animals were injected with apomorphine (1 mg/kg, Sigma) for induction of erection. During 30 minutes the presence or absence of erection was evaluated. Recovery of erectile function was defined as a visible enlargement and turgescence of the penile body (Figure 4). The χ² test was used for the statistical analysis and p < 0.05 was defined for rejection of the null hypothesis.

RESULTS

In Group I there was complete erectile response in all animals (100% – 12 in 12). On the other hand, none of the animals in Group II presented erection after the use of apomorphine.

Five of the twelve animals of Group III (41.7%) had erections after the stimulus, versus nine of the 12 animals of Group IV (75%) (Table 1).

When we compared the frequency of restoration of erection in the four Groups, Group IV showed a similar performance to that of Group I (p = 0.217), while Group III animals had a lower frequency of erections than Group I animals (p = 0.005).

Moreover, a comparison of results of Groups III and IV versus Group II showed that the frequency of erections was statistically higher in the first two groups (p = 0.037 and p < 0.001, respectively).

Finally, Group IV presented a tendency for a larger number of erections when compared to Group III (75 versus 41.7%, respectively) but this difference was not statistically significant (p = 0.098).

DISCUSSION

Restoration of sexual function after radical prostatectomy is related to the relatively slow axonic regeneration process and represents a very important clinical issue. The prolonged penile denervation leads to a progressive degeneration and fibrosis of the cavernous smooth muscle, which are associated with erectile dysfunction(18). Even with the most refined surgical techniques preserving the neurovascular bundle the complete recovery of the erectile function might take from 18 to 24 months. Thus, the faster the cavernous nerve recovery, the higher the chances of a better functional outcome after surgery.

This study showed that adult bone marrow stem cells, seeded into silicone guiding tubes, may promote
the regeneration of cavernous nerves and restore erectile function in an animal model.

Although nerve grafts have been used mostly for somatic nerves, their efficacy for autonomic nerves has already been shown. The results of the present study demonstrated a better functional outcome with the use of bone marrow stem cells and guiding tubes. Peripheral nerves have the capability of completely regenerating and reestablishing functions, at least in limited lesions. However, when a nerve is completely dissected and isolated, the guiding structure allowing adequate regrowth is lost. Thus, one could say that the underlying mechanism for an adequate repair must include a guide between both neural segments.

Based upon this, silicone guiding tubes were used to allow correct alignment and growth of the neural segments, creating a right pathway to the nerve. This is supported by the fact that, even when the guide was used without the stem cells, we found a 42% recovery in erectile function. Whenever stem cells were used, they provided trophic factors, cell adhesion molecules and extracellular matrix components, which have made the recovery even better.

Quinlan et al. (8) introduced the idea that peripheral nerve grafts could improve sexual function. Ball et al. (10) showed later that these grafts could promote the restoration of erectile function after surgery. The results of this study are highly promising and may provide the basis for the clinical application of guiding tubes lined with stem cells to restore the structure of injured cavernous nerves and the erectile function in men undergoing radical prostatectomy in the future. Such cells can be replicated in culture, seeded into guiding tubes and applied in radical prostatectomy for the restoration of cavernous nerves.

There are some practical issues surrounding the concept of cavernous nerve graft that still need to be resolved. There will be a considerable proportion of men who develop cavernous denervation and will still remain with tumor residues after prostate surgery. If these men are eligible to receive surgical grafts of silicone tubes containing stem cells from bone marrow or other matrix, such as Schwann cells that secrete neurotrophic factors (12), residual cancer cells could be stimulated by growth tumor factors released locally, thus complicating the outcome of these patients.

Although the preservation of the cavernous neurovascular bundles is the best solution to preserve the erectile function in radical prostatectomy, it is becoming increasingly common in practice to resect these beams, especially in one side, based on adverse results of biopsy unilaterally. This will encourage, predictably, an additional interest in strategies to repair the cavernous nerves. This approach would also have applications in other surgical settings such as abdominal-perineal resection and radical cystectomy.

Another potential use for nerve restoration that has not been explored is its use as a protective sheath to increase the repair of still present, but partially damaged nerves. A large proportion of men require the tincture of time to restore erectile function after a radical prostatectomy with preservation of both nerves. The use of probes or silicone molds with increased production of neurotrophic factors could accelerate the recovery of erectile function in these men.

The replacement of the cavernous nerve is a topic that has attracted the medical interest and debate. Nevertheless, new knowledge, including those generated by this study, may produce a situation of mutual gain for men undergoing surgery for prostate or other pelvic surgery and the specialists. The restoration of sexual function after pelvic operations will contribute to a better quality of life of afflicted patients and a greater satisfaction for physicians committed to the healing and well-being of their patients.

CONCLUSIONS

This study shows that adult stem cells from bone marrow, delivered in silicone guiding tubes, may promote the regeneration of cavernous nerves and restore the erectile function in an animal model in 75% of the cases.

REFERENCES