

Penile autotransplantation in rats: An animal model

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Abstract

Context: Penile allotransplantation might be a viable option for patients who need penile reconstruction.

Aims: A successful autotransplantation rat model is the first step toward proceeding for allotransplantation. We herein evaluate autotransplantation following transaction of the rat penis just distal to the urethral bulb.

Settings and Design: Experimental animal study.

Materials and Methods: Five Sprague–Dawley rats weighing 520 g (SD 19) were used. Utilizing a magnification of 6-40, transection and immediate anastomosis of the tunica albuginea, urethra, dorsal vein and nerves were carried out. Vesicostomy was made to divert urine. The glandular skin was sutured to the perineum and the abdominal wall was closed in layers.

Statistical Analysis Used: Descriptive statistics.

Results: Average surgery time was 8 h. The first two rats had no vesicostomy and died in the first postoperative day from retention. Three rats tolerated well the procedure and survived to the end point. One rat was sacrificed at day 10 and histopathology showed 30-50% necrosis of the implanted penis. Another rat was sacrificed at day 20 and showed normal cavernous tissue. The fifth rat was sacrificed 3 months postoperatively and showed evidence of moderate corporal fibrosis. Urethral fistula and necrosis of corpus spongiosum, dorsal nerve necrosis and dorsal vein occurred in all animals.

Conclusions: Penile autotransplantation in rats is feasible and provides the basis for evaluation of the corpora cavernosa in an allotransplantation model. Long-term urethral continuity and dorsal neurovascular bundle survival in this model is difficult to establish.

Key Words: Animal model, histopathology, penis, rat, transplantation

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INTRODUCTION

Early success of composite tissue allotransplantation (ALT) is gaining attention from the medical community and the

public.^[1,2] Penile ALT might be a viable option for patients who need penile reconstruction. Basic questions need to be answered before contemplating clinical application.^[3] It is not known how ALT and immunosuppression affect erectile tissue, urethra and penile growth. The rat is a suitable experimental animal in terms of availability, resistance to infection, ease of maintaining and less body weight. A successful autotransplantation rat model is the first step toward proceeding for ALT. Earlier experiments circumvented the difficulty of penile transplantation in rats by different methods. These variations included the use of an internal pudendal artery based free autograft without establishing urethral continuity, a nonvascularized allograft or an allograft with arterial anastomosis to the distal corpus spongiosum.^[4-6]

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We set out to evaluate the histopathology outcome of penile autotransplantation in rats including anastomosis of the tunica albuginea, urethra and dorsal neurovascular bundle.

MATERIALS AND METHODS

Five Sprague–Dawley rats weighing 520 g (SD 19) were used. All procedures were carried out under general anesthesia. Animals were anesthetized using Ketamine-xylazine (60 mg/kg; 7.5 mg/kg i.m., the procedure was carried out in a sterile fashion, using heparin 100 iu/kg/h i.v. 2 min before transaction and utilizing a magnification of 6-40.

A circumcision incision at the base of the glans penis was made and carried longitudinally into the lower abdominal wall [Video I]. The dorsal neurovascular bundle and urethra were dissected free from the tunica albuginea. Histologically, the dorsal arteries were 0.3-0.4 mm in diameter and were difficult to anastomose [Figure 1]. We carried out the autotransplantation without re-establishing the continuity of the dorsal arteries. Vascular clamps were applied to the dorsal vein, urethra and corpora [Figure 2]. Anastomosis of the tunica albuginea was carried out with 0-8/0-10 continuous and interrupted non absorbable sutures. The guide wire of a 22 gauge angiocath was used to temporarily stent the urethra. The urethra was anastomosed using 0-8 interrupted absorbable sutures. The dorsal vein and nerve were anastomosed using 0-10 and 0-11 non absorbable sutures. A vesicostomy was made to divert urine in the last three animals. The glandular skin was sutured to the perineum and the abdominal wall closed in layers.

The animal was transferred to an isolation cage where free access to food and water was allowed. Postoperatively for one week the animals received analgesia in the form of Ketoprofen 5.0

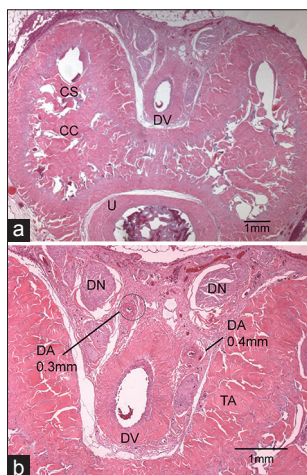


Figure 1: Cross section of the normal rat penis. (a) Two large cavernous sinuses (CS) supply the corpus cavernosum (CC). The dorsal vein (DV) and urethra (u) are shown. (b) A detail showing the small dorsal arteries (DA) and dorsal nerves (DN)

mg/kg SC q12h as needed. No antibiotic was administered. Postoperatively animals were observed daily for penile gross appearance, skin necrosis, difficulty in micturition and pain with the intention to sacrifice animals with difficult to treat conditions. Animals which completed uneventful predetermined observation periods were evaluated under general anaesthesia for urethral continuity using a guide wire; then sacrificed to obtain tissues for examination.

Animals were sacrificed at 10, 30 and 90 days. The penis was harvested and preserved for pathological evaluation. The animal was subjected to euthanasia using an overdose of ketamine i.p.

Pathological evaluation

The penile tissue was fixed in 10% formaldehyde and then processed in the pathology lab. From each specimen, six sections

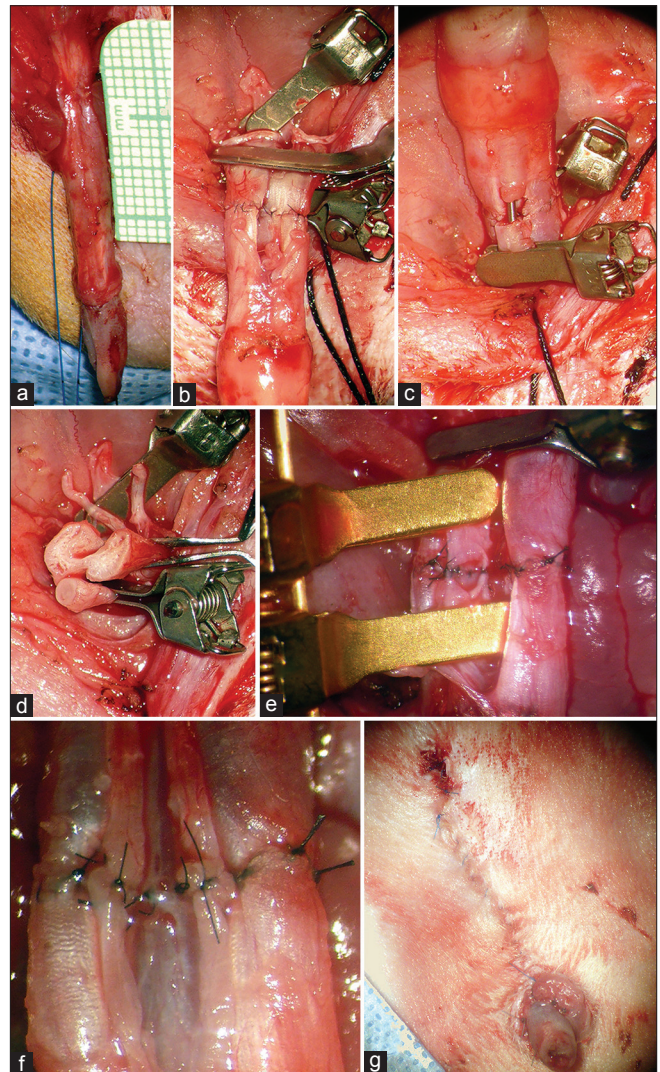


Figure 2: Surgical technique. (a) Penile dissection. (b) Vascular clamps are applied to the corpus cavernosum, dorsal vein and urethra. (c) The tunica albuginea is anastomosed. (d) Urethral anastomosis. (e) Dorsal vein anastomosis. (f) The dorsal nerves are approximated. (g) Vesicostomy

were taken and submitted for staining for hematoxylin and eosin (H and E). The sections were evaluated for the presence and degree of tissue necrosis, the development of neovascularity, healing of tissue components (vessels, nerves, cavernous tissue and urethra), presence of elastic fibers and the development of fibrosis. The method of evaluation was the subjective assessment of a single histopathologist (LA).

Outcome measures and statistical analysis

The analysis included operative time, urethral calibration and occurrence of retention, extravasation, infection, tissue sloughing, penile necrosis and mortality. Histopathological examination focused on the recovery of normal cavernous tissue, blood vessels, nerve fibres, and urethra and skin structures. Descriptive statistics are reported. Values are expressed in mean and standard deviation.

RESULTS

Average weight of rats before surgery was 516 ± 11.4 g. Surgery time was 6.8 ± 0.8 h, corpora anastomosis duration was 64 ± 4.2 min while urethral anastomosis duration was 20 ± 3.5 min. The first two rats died in the second postoperative day from clot retention.

Subsequent rats were subjected to vesicostomy to drain urine. These three rats tolerated well the procedure and survived to the end point. Calibration of the urethra showed fistula at the site of anastomosis after 10 days and narrowing and obliteration of the lumen distal to the anastomosis after 30 and 90 days respectively. The glans penis and covering skin showed necrosis. No suppuration was found in any animal.

Histopathology at day 10 showed 30-50% necrosis of corpus cavernosum of the re-implanted penis. At 30 days, the cavernous tissue and tunica albuginea were viable distal to the anastomosis [Figure 3a]. There was no histological evidence in the corpora for infarction, necrosis or fibrosis; however, there was chronic inflammation. At 90 days, there was no infarction or necrosis distal to the anastomosis; only moderate fibrosis and chronic inflammation were found [Figure 3b]. The urethra showed hyperkeratosis. The viability of the dorsal nerves or vein was not evident.

DISCUSSION

Hu *et al.* (2006) reported the first penile transplantation in humans.^[7] The graft survived well for two weeks, then was excised because of a severe psychological problem. Histopathology showed normal tissues. This case highlights the complex basic issues that need to be addressed before clinical application is started on a large scale. Three basic questions need to be addressed in analogy to the case of composite tissue

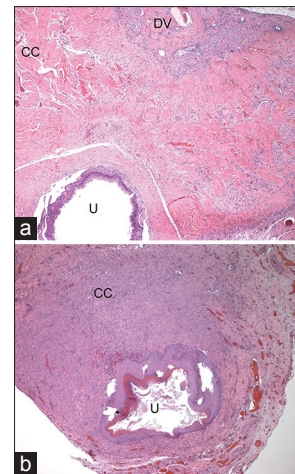


Figure 3: Histopathology of the autotransplanted penis. (a) One month. The cavernous tissue, tunica albuginea and urethra are viable. There is chronic inflammation. (b) Three months. There is no infarction or necrosis. There is moderate fibrosis, chronic inflammation and urethral hyperkeratosis

allotransplantation of the face: Can it be done, how long will the transplant last, and should it be done?^[3]

Penile tissue loss due to amputation has been treated successfully by replantation.^[4,5,8,9] The tunica albuginea and its contained vascular structures are approximated without the aid of magnification. Microvascular anastomosis is used only for the dorsal penile vessels and nerves. Long-term follow up showed good functional and aesthetic outcomes. No data are available on the effect of long-term immunosuppression on transplanted penile tissues regarding histopathology, function and growth. The rat is an attractive animal model for penile transplantation for several reasons. First, several studies have used rats to evaluate the physiology of erection, the effect of pathological condition on erection and drug response.^[10-12] Second, the small animal size allows experimentation with various immunosuppressive regimens, the cost of which is staggering high in larger animal species. Third, the rat is an economic animal, widely available, easy to maintain and resistant to infection. Several researchers contemplated the penile transplantation procedure in rats. Akyurek *et al.* performed penile autotransplantation by harvesting the penis as an island flap based on its internal pudendal blood supply and transferring it to the groin.^[4] A vascular anastomosis was made with the epigastric vessel. The transplanted penis showed normal structure at seven days in most of the animals. Transforming these encouraging results to an allotransplantation model and for a longer duration is yet to be seen. Furthermore, the nonphysiological transfer of the urethra and lack of nerve anastomosis limit the usefulness of this model for these structures. Allotransplantation in the rat was carried out by a non-vascular anastomosis technique.^[5] The donor penis was wrapped in the omentum of the recipient animal and left intra-abdominal for several weeks. Exploration

after three weeks revealed that neovascularity has developed and supplied the implanted penis. The authors suggested but did not show that the penis may be transferred based on its new blood supply to the perineum.

Penile autotransplantation in rats is technically challenging but feasible. The down side in our model is that dorsal artery anastomosis is not possible, the long-term viability of the distal urethra and glans is poor, the generation of dorsal nerves is not evident and the loss of penile skin due to necrosis is found. It remains that the model is suitable to evaluate only corpora cavernosa for viability and subsequently function. The development of chronic inflammation and fibrosis further complicate this goal. The compromise of blood supply to the urethra is probably due to lack of circulation through the corpus spongiosum at the site of anastomosis. We used interrupted sutures passing through the whole thickness of the urethral mucosa and corpus spongiosum. A better method is probably to anastomose the mucosa and tunica covering of the corpus spongiosum separately allowing the circulation to pass across the anastomosis. The glans penis as a consequence suffers from ischemia due to lack of urethral and dorsal artery supply. The glandular skin suffers from absence of collateral circulation from the surrounding skin and therefore subject to necrosis. These caveats have been circumvented in other reported experiments by establishing an arterial to distal corpus spongiosum anastomosis.^[6] While this technique provides adequate arterial supply to the urethra, glans and covering skin, it does not resemble normal anatomy or physiology.

CONCLUSION

Penile autotransplantation in rats is feasible. The technique may provide a viable animal model basic to research evaluating penile allotransplantation, studying the histopathology of corpora cavernosa and its possible functions. However,

further refinements of the technique need to establish urethral continuity and long-term nerve re-growth.

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