Study of Clinical Practical Model of Urinary System Injury

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Abstract

Background: In order to improve the clinical treatment level of urinary system injury, it is necessary to build up an animal model of urinary system wound, which is not only analogous to real clinical practice, but also simple and practical.
Methods: We have developed the third generation of firearm fragment wound generator based on the first and the second producer. The best explosive charge of the blank cartridge was selected by gradient powder loading experiments. The firearm fragment injuries were made to the bulbous urethra of 10 New Zealand male rabbits. One week preoperatively and 2, 4 and 8 weeks postoperatively, all the animals underwent urethroscopy and urethrography. At 2, 4 and 8 weeks postoperatively, two animals were randomly selected and killed, and the urethra was cut off for pathological examination.
Results: The shooting distance of the third generation of firearm fragment wound generator is 2 cm. The best explosive charge of the blank cartridge is 1 g of nitrocotton. All rabbits survived the procedures and stayed alive until they were killed. Injuries were limited to bulbous urethra and distal urethra. Round damaged areas, 1–1.5 cm in length, on the ventral wall were observed. Ureteroscopy results showed that canal diameter gradually shrank by over 50% in 9 rabbits. The rate of success was 90%. Urethrography result noted that a 1–1.3 cm stricture was formed at the bulbous urethra. Histology results of injured stricture urethra showed that fibrous connective tissue hyperplasia and hyaline degeneration caused further stricture in the canal.
Conclusions: The third generation of firearm fragment wound generator imitates the bullet firing process and is more accurate and repeatable. The corresponding rabbit model of traumatic complex urethral stricture simulates the real complex clinical conditions. This animal model provides a standardized platform for clinical researches on treating traumatic injuries to the urinary system.

Key words: Animal; Injury; Models; Urogenital System; Wound Generator

Introduction

Ever since the World War Ⅱ, local wars, military confrontations, and terrorist activities keep emerging one after another. Among all varieties of traumatic injuries, injuries to the urinary system account for 2–4%, most of which are induced by firearm fragment.[1-3] With a view to enhancing treatment efficacy of urinary system war injury, a feasible and reusable animal model that simulates modern war environment is requested. Between 2006 and 2013, we have developed the 3rd generation of firearm fragment wound generator based on the 1st generation of directional shooting bombing instrument[4] [Figure 1a] and the 2nd generation of firearm fragment wound producer[5] [Figure 1b]. And we also established corresponding animal model.

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Methods

Ethics

All animal experimental protocols were approved by the committee of animal research at the PLA General Hospital and the PLA 309 Hospital, and the procedures were performed in accordance with guidelines for humane handling of animals.

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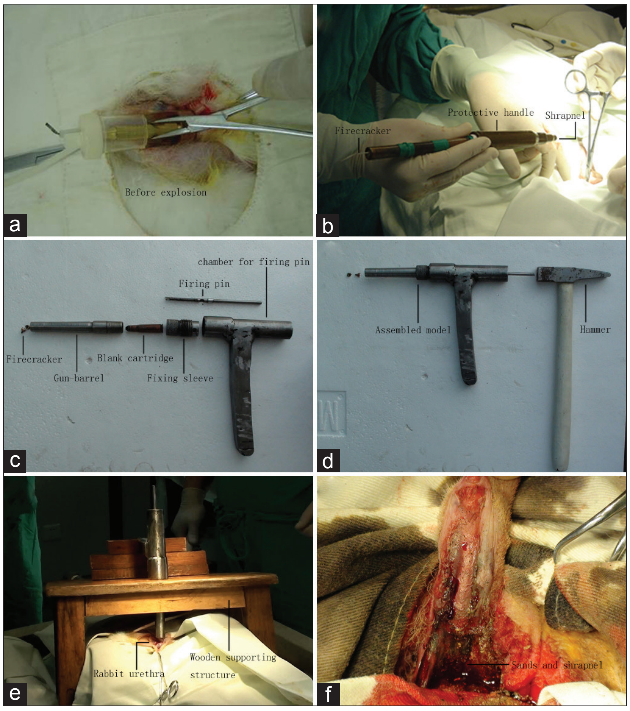

Figure 1: The three generation of firearm fragment wound generator. (a) The first generation of directional shooting bombing instrument; (b) The second generation of firearm fragment wound producer; (c-f) The third generation of firearm fragment wound generator ([c] Components of the device; [d] Assembled device; [e] Aiming at the bulbous urethra with the help of the wooden supporting structure; [f] Complex traumatic urethral injury).

The third generation of firearm fragment wound generator

Simulates rifle shooting bullets. The generator consists of hammer, needle, blank cartridge, rifle tube, and wooden fixer. As the hammer hits the needle, the needle hits the bottom of blank cartridge, thus igniting the explosives inside the bullet. Fire and shock wave shoot out of rifle tube and eject fragment, which is plastered on the muzzle, thus causing firearm fragment wound to the animals. Wooden fixer helps to stabilize the generator for precise targeting. The distance of shooting is 2 cm according to the height of the wooden fixer [Figure 1c-e].

The stand explosive charge of blank cartridge of 81–1 automatic rifle is 2.98 ± 0.02 g of nitrocotton (explosion speed 1500 m/s). In order to find the best explosive charge for this study, we recharged some blank cartridges with 0.5, 1, 1.5, 2, 2.5 and 3 g of nitrocotton, respectively. Then the isolated urethra of New Zealand male rabbit was shot using these blank cartridges with different explosive charge, and the best explosive charge was selected.

The third generation of the rabbit model of traumatic complex urethral stricture

Animal experiments were carried out in the Experimental Animal Centre at the General Hospital of PLA in China. Twenty New Zealand male rabbits ranging from 2.8 to 3.2 kg were selected. The animals were randomly divided into two groups, Group A (firearm fragment wound) and Group B (normal control), with 10 rabbits in each group. Rabbits in Group A were anesthetized with ketamine hydrochloride intramuscular injection (0.3 ml/kg) and pentobarbital intravenous drip (25 mg/kg). Rabbits were placed in a supine position. Membrane joining penis and anus were cut through so that bulbous urethra and distal urethra were properly exposed. Rifle tube was fixed above urethra by wooden fixer and targeted at urethra 2 cm away. Firing the generator caused severe injuries. Then, wounds were wrapped in wet gauze and left for 30 min. After that, debridement was performed. The necrotic tissue was cut off and 6-0 absorbable suture was used for anastomosis to restore the urethral continuity. Anti-infection treatment was provided postoperatively. All surgeries were performed by the same surgeon.

One week preoperatively and 2, 4 and 8 weeks postoperatively, all the rabbits underwent urethroscopy and urethrography. At 2, 4 and 8 weeks postoperatively, two rabbits of Group A and two rabbits of Group B were randomly selected and killed by overdose of anesthesia after the examination, and the urethras were cut off for pathological examination.

Results

The third generation of firearm fragment wound generator

The operation of the home-made device is safe and easy. The wooden fixer can improve the stability of shooting. And the firing accuracy is 100% when the shooting distance is 2 cm. With the help of the gradient powder loading experiments, we found that the firing power was not enough when the explosive charge was 0.5 g of nitrocotton, observing that only the parietal layer of urethra was hurt while the urethral lumen was not effectively damaged. On the other hand, the explosive was so great that the fragment could penetrate the whole body and led to large defect of the urethra when the explosive charge was more than 1 g of nitrocotton. Hence, the best explosive charge of the blank cartridge is 1 g of nitrocotton, with which the firing power is under control, and the full-thickness urethral damage can be made.

The third generation of the rabbit model of traumatic complex urethral stricture

The shooting process was safe and successful. All rabbits survived the procedures and stayed alive until they were killed. In rabbits of Group A, injuries were limited to bulbous urethra and distal urethra. Round damaged areas, 1.0–1.5 cm in length, on the ventral wall were observed; coking, soiling and bleeding were observed within the injured area where identifiable firearm residues and fragments were observed. Inflammation and semi-coking were found on the edge of injured urethral wall and complete damage in the central area. Dorsal wall was found with membranous inflammation and erosion as well as slight bleeding [Figure 1f]. Surrounding tissue and skin were well preserved. Hematuria was observed in 4 postoperative rabbits and disappeared 3–5 days later. One rabbit was found with excessive loss of urethra tissue which leads to urinary fistula 1 week postoperatively.

Using urethroscopy, urethral stricture was found in nine rabbits 2 weeks after the operation. Its inside diameter shortened more than 50% in the narrow place. Dorsal wall was found with remaining rough spots of mucosa and erosion as well as slight bleeding. Obvious necrotic tissue rising into the cavity could be seen on ventral wall. Urethroscopy failed in one rabbit because of the urinary fistula. The urethral stricture was found worse 4 weeks than 2 weeks after the operation, and polypoid lesion could still be observed on the ventral wall. Eight weeks after the injury, urethroscopy revealed the most serious urethral stricture with a pale scar around in the urethral channel [Figure 2b].

Retrograde urethrography showed that the length of the narrow segment of the bulbous urethra and distal urethra was 1.0–1.3 cm and the diameter decrease was more than 50% 2 weeks after the trauma. Four weeks and 8 weeks later, the length of the narrow segment remained unchanged while the degree of stenosis got worse [Figure 2d].

Two weeks after the operation, obvious stenosis of the urethra was observed under the HE slices. Tissue hyperemia, edema and epithelial cells shedding occurred in the injured wall of the urethra. The epithelial cells were pressed to be spindle and parallel to the surface of the urethra. Swelling and vacuolar degeneration were seen in the basal cells. More collagenous fibers were seen in the submucosa. Powder or metal particles were seen in the smooth muscle layer of urethra around the injured area. Microscopically, the powder or metal particles were black or brown needly crystal or crystalline powder. The smooth muscle layer was almost replaced by collagenous fibers, and some of the collagenous fibers were hyaline degeneration. Cluster of neutrophile granulocyte was seen in the lumen of small vessel. Four weeks after the operation, the stenoses of the urethra were more severe. The lymphocyte and neutrophile granulocyte infiltration was seen in the mucosa and submucosa. Urothelium metaplasia to columnar epithelium or squamous epithelium could be seen. In the mucosa and submucosa, much fibrous tissue was seen, especially around the particles. Eight weeks after the operation, the lumen of the urethra was tiny. A few lymphocytes were seen in the mucosa. Moderate hyperplasia and atrophy were all seen in the urothelium. In the submucosa, more fibrous tissue were seen [Figure 2f].

The results of urethroscopy, urethrography and histology in Group B were nomal [Figure 2a, c, e].

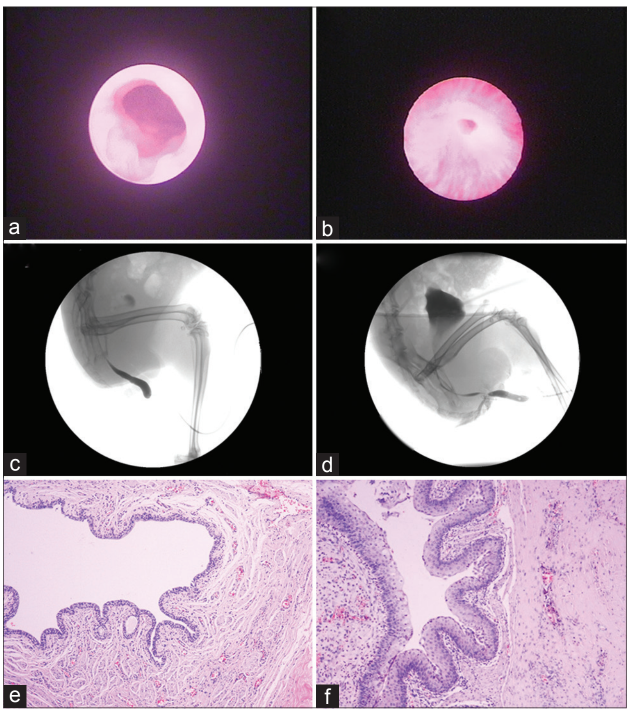

Figure 2: Results of examinations 1 week before operation and 8 weeks after operation. (a, c, e) Normal urethra 1 week before operation ([a] Urethroscopy; [c] Urethrography; [e] H and E, ×100); (b, d, f) Complex urethral stricture 8 weeks after operation ([b] Urethroscopy; [d] Urethrography; [f] H and E, ×100).

Discussion

Firearm injury is generally caused by burning gunpowder or explosion that causes projectiles to shoot and, therefore, damage the targets. Firearm fragment wound is a common type of firearm injury.

Between 2006 and 2012, we concentrated on manufacturing trauma-generator and making animal model of urinary system war injury and kept improving. One should note that on one hand, China is subject to strict weapon control regulations; on the other, direct gun shooting will cause complicated injuries, which may slip beyond control. Therefore, we try to use home-made devices to simulate gun shooting and we have established animal models for urethral injury [Table 1].

The first generation device is based on directional explosion, causing urethral stricture. Therefore, the device is simple in design, safe in operation, steady in explosion, and precise in targeting. The device is able to control the degree of injuries and scope thereof. Long-term observation results found urethral strictures in a stable state and thus it could form an ideal animal model for research on treating urethra injured by explosion. However, due to the widespread usage of explosive killing weapons in modern battlefield, firearm fragments have become the major injury source.[1] Yet, the first generation device is only capable of mimicking gunpowder explosion, but not injuries induced by firearm fragments, and such limit is obvious.

In the second generation of firearm fragment wound producer, firecrackers exploded in the first explosion section. As a result, shockwaves were generated and passed through the second, third, and fourth explosion sections as well as the sight section, thus projecting the fragments shooting the targets. Simple as it might be, this exactly simulates the actual process of generating firearm fragment.

Table 1: Three generation of war wound generators and corresponding animal models of war injury

Items	1st generation	2nd generation	3rd generation
Name	Directional shooting bombing instrument	Firearm fragment wound producer	Firearm fragment wound producer
Time	2006	2009	2013
Explosive	Firecracker contains regular explosives totaling (234 ± 3.84 mg)	Firecracker contains 3 g black powder (explosion speed 400 m/s)	Blank cartridge contains 1 g nitrocotton (explosion speed 1500 m/s)
Shooting distance	0	1 cm	2 cm
Firing mode	Lit the fuse	Lit the fuse	Hammer hits the needle
Type of trauma	Bomb injury	Firearm fragment injury	Firearm fragment injury
Animal	New Zealand male rabbits	Beagles	New Zealand male rabbits
Trauma location	Bulbous urethra	Mid-ureter	Bulbous urethra
Animal model	Rabbit model of bombing wound urethral stricture	Beagle model of ureteral war injury	Rabbit model of complex urethral stricture
Advantage	Simple to make, easy to handle	Relative simple to make, feasible and efficient	Imitate bullet firing process, more accurate and repeatable
Disadvantage	Only imitate gunpowder bomb injury	Less valuable in aiming and fire control	Complex to make
Research based on the animal model	Biodegradable urethral stents[6] (seeded with autologous urethral epithelial cells[7,8]) in the treatment of posttraumatic urethral stricture	Biodegradable polylactic acid ureteral stent application for treatment of ureteral war injury[5]	

The Beagle dog model with firearm fragment wound is proved to be feasible in that it efficiently simulates the generation of firearm fragment wound and the whole development process. However, the device suffers with a low success rate in the first try, due to insufficient precision in targeting and power control.

The third generation device simulates injuries induced by firearm fragments and the design closely mimics real guns. Wooden fixer helps stabilize the device for more precise targeting. The process of firing is by knocking the firing pin manually each time instead of auto-fire system, which is not only safe and stable, but also meet the Chinese regulations on guns and weapons. Gradient powder loading experiments were carried at a variety of ranges, and most effective loading volume has been identified.

The firearm fragment injury was made to the rabbits' urethra. Then, wounds were wrapped in wet gauze and left for 30 min because it takes about 30 min for wounded soldiers to be transferred from the battlefield to division aid stations or a field hospital for treatment.[1]

We then followed the principle of conventional treatment for urethral firearm fragment injury, debridement and suturing of the wounds to restore the continuity of the urethra. One animal is found with urinary fistula. The remaining 9 animals are found with thread-like urethral stricture 8 weeks postoperatively. Stricture length ranges between 1 and 1.3 cm (the total length of rabbit urethra is about 8 cm). Modeling success rate is 90% ($P < 0.05$).

In addition to the directly wounded section of urethra, which was cut-off, the surrounding tissues where urethrourethrostomy had been performed were also contused and stricken by shockwaves. Besides, due to the firearm fragments and the gunpowder polluting the surrounding tissues, damages caused by the wound-cleansing process, and the irritation caused by gunpowder residues at the wounded sites, sands as well as absorbable suture, the inflammation was caused where urethrourethrostomy had been performed. Secondary hyperplastic scars kept growing, and urethrostenosis got worse.

From the histological view, at 2 weeks postoperatively, tissue necrosis, congestion and edema were found where urethra was damaged. At 4 weeks, postoperatively, invasion of inflammatory inflamed cells and hyperplasia epithelium were found where urethrourethrostomy had been performed. At 8 weeks postoperatively, hyperplasia of fibroblast was found in the whole layer. From the histological view, this is strong evidence proving that hyperplastic scar and ureterostenosis existed.

The findings show that the device in discussion is safe and effective. The length of traumatic urethral stricture of rabbits ranges between 1 and 1.3 cm (the total length of rabbit urethra is about 8 cm), while length of complex urethra stricture of human being ranges from 2 to 3 cm (the total length of male urethra is ranging between 16 and 22 cm). Hence, the animal model of traumatic complex urethral stricture caused by explosion fits Campbell definition for complicated urethra stricture.

In conclusion, the third generation of firearm fragment wound generator imitates the bullet firing process and is more accurate and repeatable. The corresponding rabbit model of traumatic complex urethral stricture simulates the real complex clinical conditions. This animal model provides a standardized platform for clinical researches on treating traumatic injuries to urinary system.

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