



Induction of Contraception by Intraepididymal Sclerotherapy

Hyoung Keun Park¹, Sung Hyun Paick¹, Hyeong Gon Kim¹, Yong Soo Lho¹, Sang Rak Bae²

Department of Urology, ¹School of Medicine, Konkuk University, Seoul, ²The Catholic University of Korea, Uijeongbu St. Mary's Hospital, Uijeongbu, Korea

Purpose: The objective of the present study was to evaluate the efficacy of a sclerosing solution for inducing epididymal occlusion in male rats.

Materials and Methods: Male Sprague-Dawley rats were divided into two groups: an injection group (n = 20) and control group (n = 20). Before injecting the sclerosing agent, seminal vesiculectomy and sperm identification using electrostimulation were performed in all of the rats. In the injection group, 0.2 mL of 0.1% sodium tetradecyl sulfate solution was injected into the epididymis. In the sham group, only the identification of the epididymis was performed. At 4 and 12 weeks after the injection, semen was collected by electrostimulation and evaluated to assess the contraceptive effect. Epididymis was evaluated by hematoxylin and eosin (H&E) staining.

Results: After 4 and 12 weeks, semen collection was performed in the two groups. Sperms were not observed in the injection group, while there was no change in the sperms in the sham group. H&E staining showed the obstruction of epididymal tubules and an accumulation of inflammatory cells in the injection group.

Conclusions: This study showed that the sclerosing agent induced sterilization in male rats. This result suggests that the injection method can replace vasectomy as a contraceptive method. However, a further study of large animals and a clinical study are needed. Further, the long-term effectiveness of this method needs to be studied.

Key Words: Contraception; Male; Rats; Sterilization

INTRODUCTION

Vasectomy is a simple and popular surgical procedure that is used as a permanent birth control method for men. During the procedure, the *vasa deferentia* of a man are taken out and then, tied or sealed to prevent the sperm from entering into the semen. This is a widely used birth control method for men because of its high success rate and low

complication rate. However, the fear of skin incision or cutting of the *vasa deferentia* and the male genital organs prevents many men from undergoing vasectomy. Therefore, several studies [1-3] have been conducted on inducing sterilization without surgery. In these studies, injection therapy inducing vasal or epididymal occlusion has shown some efficacy, but the results have been inconsistent.

Although previously, the ligation and removal of en-

Received: Apr 25, 2014; Revised: Jun 5, 2014; Accepted: Jun 18, 2014

Correspondence to: Sang Rak Bae

Department of Urology, The Catholic University of Korea, Uijeongbu St. Mary's Hospital, 271 Cheonbo-ro, Uijeongbu 480-717, Korea.
Tel: +82-31-820-5354, Fax: +82-31-847-6133, E-mail: robinbae97@catholic.ac.kr

gorged veins was the most widely used surgical method for varicose veins in the legs, with the development of various occlusive agents, simple injection therapy now plays a major role in the treatment of varicose veins [4-6]. The mechanism of action of an occlusive agent is the induction of an epithelial cell injury and fibrosis and finally, the occlusion of the vein lumen [7].

As the lumen of the epididymis is lined by epithelium similar to that in veins, the occlusive agent is expected to have a similar effect, that is, luminal occlusion. If the drug has a similar occlusive effect on the *vas deferens* or epididymis, this injection therapy could replace vasectomy.

The objective of the present study was to evaluate the safety and efficacy of a sclerosing solution, sodium tetradecyl sulfate (STS) for inducing epididymal occlusion in male rats.

MATERIALS AND METHODS

We used a total of 40 male Sprague-Dawley rats (6-week-old male rats) in this study. All the animal experiments were conducted according to the guidelines of the Animal Institutional Review Board of Konkuk University. These rats were selected because of the easy access to the epididymis and semen collection.

Twenty rats were used as controls, and 20 rats were included in the injection group, in which sterility would be induced by using the STS solution.

Two weeks before semen collection, the seminal vesicles were excised via a midline incision after anesthesia.

An electrostimulation method was used for semen collection, as described in detail in a previous study [8]. With

a transrectal probe (60 Hz, 3 V, 0.5 A), sine-wave electrostimulation was applied to the rectum of an anesthetized rat. The urethral meatus was seen when the prepuce was retracted. The semen found at the meatus was collected by a pipette, and the presence of sperms was confirmed under a microscope.

Induction of ejaculation was achieved in all of the rats, and the presence of sperms in the semen was confirmed in these rats (Fig. 1B).

After an analysis of the semen, 0.2 mL of 0.1% STS solution was injected into the epididymis with a 24 G syringe in the rats of the injection group. Under general anesthesia, we exposed the rats' epididymis and injected about 0.2 mL of the STS solution in both the head and the tail of the epididymis (a total of 0.4 mL on one side). The epididymis of the rats in the control group was identified, but no additional procedure was performed. There was no difficulty in injecting the sclerosing agent, and after injection, the testis and the epididymis were pulled back into the scrotum.

After 4 weeks and 12 weeks of injection, the 2nd and the 3rd semen collections were performed by the electrical stimulation method. The presence of sperms was evaluated by two examiners who were unaware of the group identity.

Autopsies were performed shortly after taking the final semen sample. The epididymis was excised and fixed in 4% neutral buffered formalin and subjected to paraffin embedding for further sectioning. Hematoxylin and eosin (H&E) staining was performed to detect any structural anomaly of the epididymis.

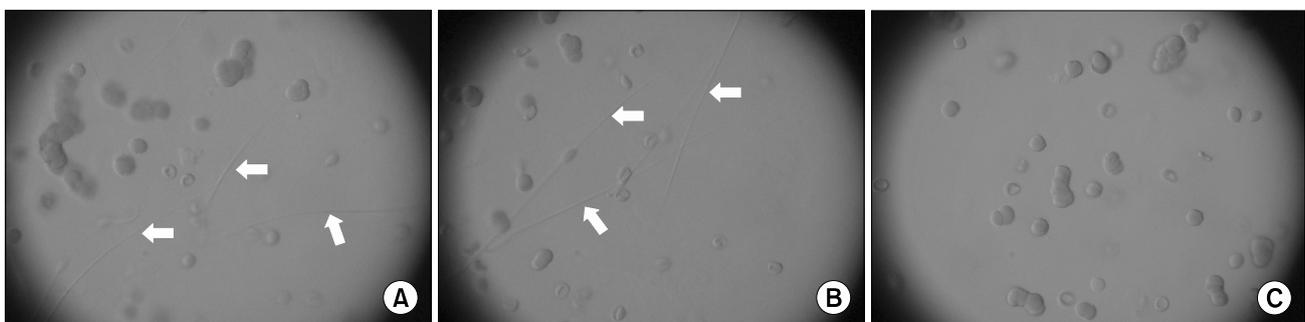


Fig. 1. (A) Sperm identification before injection. The white arrows indicate the sperm of the rat. (B) Sperm observed in the control group. The white arrows indicate the sperm of the rat. (C) No sperm observed in the injection group (A~C: simple staining, $\times 100$).

RESULTS

1. Semen analysis

After 4 weeks and 12 weeks of injection, the induction of ejaculation was achieved in both the groups.

The rats of the control group showed sperms in the semen (Fig. 1B). However, no sperms were observed in the semen of rats from the injection group (Fig. 1C). There were no cases of testicular atrophy or swelling in the two groups.

2. Pathology

The epididymis of the rats in the injection group was slightly swollen as compared to that of rats in the normal control group. However, there was no abscess formation.

H&E staining showed an infiltration of inflammatory cells in the epididymis of the rats in the injection group. The epididymis was swollen grossly, and leukocytes were infiltrated in the epididymal tubules and stroma. An obstruction of the epididymal tubules was observed in the rats of the injection group. Inflammation or obstruction of the epididymal tubules was not seen in the rats of the normal control group (Fig. 2).

DISCUSSION

Vasectomy has been proved to be a safe and efficacious method of male sterilization.

However, the thought of someone operating on their

genital organs generates fear in many men, and this is the main reason why men do not want to undergo vasectomy. Therefore, the induction of sterilization by injection and without surgery could be more acceptable to these men.

The mechanism of action of injection therapy is that when a drug is injected into the epididymis or the *vas deferens*, it occludes the epididymal lumen or the *vas deferens* and prevents sperm movement. Another mechanism of action of injection therapy is the prevention of sperm formation in the testis when a drug is injected into the testis. As zinc-based solutions have been effective in the prevention of sperm formation in the testis [9], a zinc-based solution, Neutersol[®], is now used as an agent for sterilization, and the Food and Drug Administration (FDA), USA, has approved its use for the sterilization of dogs. However, it has not been approved for male sterilization, and there is no report about its long-term effect on the testis.

In contrast to testicular injection that induces the prevention of sperm formation, epididymal or vasal injection therapy has not been used widely. Fahim et al [2] reported a sterilization rate of 100% in 15 dogs that received an epididymal injection of zinc arginine. However, a study by Singh et al [3] did not show similar effectiveness after vasal injection. Thus far, the FDA has not approved any agent that induces the occlusion of the epididymis or the *vas deferens*.

Sclerotherapy has been used for centuries to treat spider veins. The technique involves the injection of a chemical into the veins.

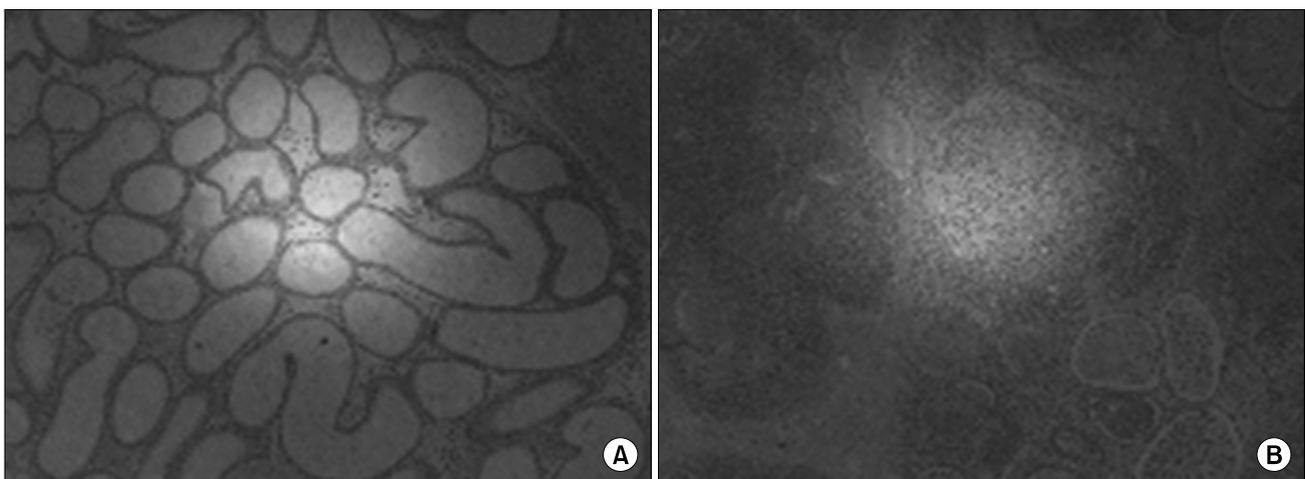


Fig. 2. (A) A normal epididymal tubule in the control group. (B) Obstruction of epididymal tubules was observed in the injection group (A, B: H&E, $\times 200$).

Sclerosants are injectable chemical agents that can scarify and obliterate the vascular tissue. A liquid or foam sclerosing agent is injected into the vein to cause localized damage to the inner lining (endothelium) of the vein. This leads to inflammation, collapse, and thickening or scarring of the vessel. STS is a widely used sclerosant for the treatment of telangiectatic veins and has been approved for use in the United States by the FDA.

We assumed that this sclerosing agent could cause damage to the epididymal endothelium and may cause obliteration as in the veins.

This study showed that the injection of STS into the epididymal tubules induced sterilization in male rats and that the sterilization was achieved due to the occlusion of the epididymal tubules. This is the first study to show that STS may be an effective agent for male contraception.

After performing more studies, this injection therapy could be used for an induction of sterilization in dogs and cats that need to undergo vasectomy or orchiectomy. Moreover, after performing clinical trials, we expect this injection therapy to play an important role in male contraception.

This study has several limitations. First, we determined that there were no sperms in the semen at only 4 and 12 weeks. The efficacy of the sclerosing agent is reported to be more than 90%, but re-canalization occurs sometimes in the great veins such as the saphenous vein [10]. As continuous blood flow differs from an intermittent small amount of vasal fluid, there is a much lower possibility of recurrence. However, this must be evaluated by a large long-term study.

Further, a confirmation of the absence of pregnancy after mating is needed to further strengthen these results.

Secondly, epididymal tubular damage-related pain or inflammation needs to be evaluated to apply these results in a larger animal or human study.

This study is a preliminary short-term study of rats. Therefore, a further study of large animals and a clinical study are needed. Moreover, the long-term effectiveness of this method needs to be studied.

Despite these limitations, this study is meaningful as it shows that a simple and effective method of contraception without surgery is possible.

CONCLUSIONS

This study demonstrated sclerosing agent-induced sterilization in male rats. The results suggest that an injection method could replace vasectomy as a contraception method. However, this study is a preliminary short-term study of rats. Therefore, a further study of large animals and a clinical study are needed. Moreover, the long-term effectiveness of this method needs to be studied.

ACKNOWLEDGEMENTS

This work was supported by National Research Foundation of Korea (Project number: NRF-2010-0004955).

REFERENCES

- Oliveira EC, Moura MR, Silva VA Jr, Peixoto CA, Saraiva KL, de Sá MJ, et al. Intratesticular injection of a zinc-based solution as a contraceptive for dogs. *Theriogenology* 2007;68:137-45.
- Fahim MS, Wang M, Sutcu MF, Fahim Z, Youngquist RS. Sterilization of dogs with intra-epididymal injection of zinc arginine. *Contraception* 1993;47:107-22.
- Singh SK, Rovani E, Frick J. Intravasal application of BCG (Bacille Calmette Guerin) is not an effective method for control of fertility in the male: a preliminary report. *Contraception* 1993;47:303-6.
- Leach BC, Goldman MP. Comparative trial between sodium tetradecyl sulfate and glycerin in the treatment of telangiectatic leg veins. *Dermatol Surg* 2003;29:612-4.
- Bowes LE, Goldman MP. Sclerotherapy of reticular and telangiectatic veins of the face, hands, and chest. *Dermatol Surg* 2002;28:46-51.
- Campbell B. Varicose veins and their management. *BMJ* 2006;333:287-92.
- Green D. Sclerotherapy for the permanent eradication of varicose veins: theoretical and practical considerations. *J Am Acad Dermatol* 1998;38:461-75.
- Paick SH, Lee BK, Yoon SW, Baek M, Kim HG, Song EY, et al. Electroejaculation in the male rat. *Korean J Urol* 2008; 49:266-70.
- Oliveira EC, Moura MR, de Sá MJ, Silva VA Jr, Kastelic JP, Douglas RH, et al. Permanent contraception of dogs induced with intratesticular injection of a Zinc Gluconate-based solution. *Theriogenology* 2012;77:1056-63.
- Pannier F, Rabe E; for the Guideline Group. Results from RCTs in sclerotherapy: European guidelines for sclerotherapy in chronic venous disorders. *Phlebology* 2014;19: 29(1 Suppl):39-44.