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Assessment of an Intraprostatic Injection Technique through a Perineal Approach in Macaques

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We developed a surgical procedure for accessing the prostate gland of the cynomolgus monkey (*Macaca fascicularis*) through the perineal cavity. The procedure can be used for direct injection of compounds into the prostate gland and (or) for the collection of biopsies. The rationale for developing this technique at our site was the need for precise injection into the gland with a low probability of error, as the compound tested in a subsequent study required prostate-specific antigen for activation. A perianal incision was made approximately 1 cm ventral to the anus, and the muscle and subcutaneous tissue were bluntly dissected between the urethra and the rectum. The prostate gland was easily visualized after dissection, and could be grasped gently by the capsule and exteriorized through the incision, thus allowing easy access to the prostate for study purposes. On the basis of mock injections with methylene blue dye and gross observation of prostate tissue at necropsies immediately after injection, we recommend that 2 injections be given per lobe of prostate, and injections should be to a depth of 2 to 3 mm to provide uniform distribution of injected compounds. To minimize back pressure and leakage from the injection site, a small-gauge needle (23-27 gauge) should be used and the needle held in place for approximately 30 s before withdrawal. Injection volumes 64 μl per g prostate or less did not cause the back flow of methylene blue dye into the seminal vesicles.

Nonhuman primates have been used as models to study diseases and other physiologic conditions of the prostate gland that affect humans. Such studies often require direct access to the prostate gland for injection of prospective therapeutics and (or) for the acquisition of biopsies. A surgical procedure is presented for direct visualization of and access to the prostate gland via the perineal cavity in cynomolgus monkeys (*Macaca fascicularis*). The procedure overcomes some of the difficulties, imprecision, and trauma that are often encountered with ultrasound and other surgical (transabdominal) approaches. Many different prostatic approaches have been successful in humans and animals; however, we specifically were interested in ensuring that the injections were deposited accurately into the prostatic parenchyma, which can be difficult to achieve without direct visualization of the gland or experience with the various techniques. For example, laparoscopic surgery is an emerging technique requiring less tissue manipulation than our procedure and faster postprocedural recovery; however, the requirement for additional training and expertise precluded its use in our facility. In addition, we provide recommendations regarding the fluid volumes and procedures used for intraprostatic injection.

Materials and Methods

Animals, housing, and feeding. Three male cynomolgus monkeys (*Macaca fascicularis*) from Charles River Laboratories, Preclinical Services Nevada (Sparks, NV) colony were used in the study. The animals were of Chinese origin, captive-bred, and imported from Yunnan Province National Primate Center in south central China. The weights of these animals ranged from 2.9 to 5.4 kg, and ages ranged from 5 to 7 y. Approval to use these animals was obtained from the Institutional Animal Care and Use Committee of Charles River Laboratories, Preclinical Services, Nevada. Animals were housed individually in stainless-steel cages and the room environment maintained to meet the specifications of the US Department of Agriculture Animal Welfare Act (9 CFR, Parts 1, 2, and 3) and as described in the *Guide for the Care and Use of Laboratory Animals*.[6] Purina Primate Diet (certified; Richmond, IN), or its equivalent, was provided daily in amounts appropriate for the size and age of the animals and supplemented with fruits, cereal, vegetables, and other treats as part of the environmental enrichment program.

Anesthesia and surgical preparation. The animals were fasted overnight prior to surgical procedures. They were sedated with an intramuscular injection of ketamine hydrochloride (10 mg/kg; Ketaset, Fort Dodge Laboratories, Fort Dodge, IA). Additional anesthesia was administered through intravenous injection of ketamine (10 mg/kg; Ketaset, Fort Dodge Laboratories) and diazepam (0.50 mg/kg; Valium, Abbott Laboratories, North Chicago, IL) to maintain adequate anesthesia throughout the surgery and postsurgical procedures. Lidocaine (2%, USP injection, Bimeda, Riverside, MO) was used to provide local anesthesia at the surgical site. The animals were monitored for heart rate, body temperature, respiration, oxygen saturation, and blood pressure during surgery. The anesthetized animals were placed face down and secured on a surgical table with the legs extended over the end of the table towards the surgeon. The table was slightly inclined to elevate the perineal area. The tails were secured dorsally to avoid contamination of surgical site. The area of incision was clipped, disinfected with iodine scrub (Purdue Frederick, Stamford, CT) and 70% isopropyl alcohol (Henry Schein, Melville, NY), and covered with sterile towels. An anal purse-string suture was placed with nonabsorbable monofilament material to prevent fecal contamination of surgical site.

Perineal surgical procedure. A small skin incision (approxi-

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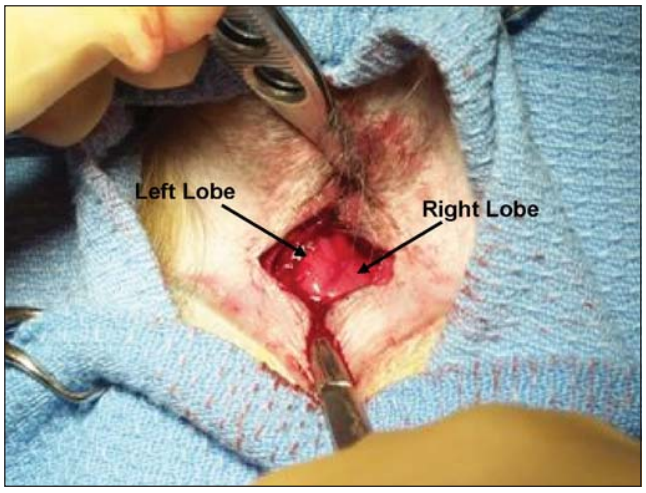

Figure 1. Dorsal view of the prostate gland after exteriorization through the perineum.

mately 2 to 4 cm) was made with a #15 blade in the perineal region approximately 1 cm below the anus. The perineal musculature involved in the dissection included the external anal sphincter, bulbocavernosus, ischiocavernosus, levator penis, and sphincter erethrae membranaceae. All of these muscles are innervated by branches of the pudendal nerve.[3] Superficially, the external anal sphincter was located directly after skin and subcutaneous tissue incision. The muscle fibers were separated gently with hemostats to maintain minimal disruption to the muscle. Once through these fibers, the surgery consisted of blunt dissection of connective tissue dorsal to the other perineal muscles previously mentioned. Careful clearance of connective tissue with hemostats overlying (dorsal) the prostate was done for better visualization and movement of the gland. The landmark used to identify the prostate was the rectum. It consistently was located immediately adjacent (ventral) to the rectum approximately 2 to 4 cm from the incision site. The seminal vesicles were not visible during our procedures. The capsule of the gland was grasped with tissue forceps caudally. The gland needed to be released from its fibrous attachment via the puboprostatic ligament and then was easily translocated to the incision site for visualization and injection (Figure 1). This translocation was done with gentle but steady pulling of the gland caudally with forceps and the surgeon's finger. For evaluation of injection volumes, a 100-µl Hamilton syringe with a 2 1/2 in., 22-gauge needle was used for injections in the 1st animal (MF2626M) and 300-µl disposable plastic syringes (Terumo U-100 Insulin syringes, 0.3 ml, 29-gauge × 1/2 in. needles, Elkton, MD) were used on subsequent animals (MF10263M and MF26162M). Single injections of methylene blue (histology grade) were made into each lobe of the prostate gland in the first 2 animals (MF2626M and MF10263M), and 2 injections per lobe of the prostate gland were made in the 3rd animal (MF26162M), to measure the effect of single and multiple injection and injection volume on the distribution of methylene blue dye. Needle placement was directed into the center of each lobe in an attempt to ensure adequate access to stromal and lumenal portions of the gland. The lobes were differentiated easily for injection purposes. A subsequent study using this technique for prostate toxin injection revealed consistent microscopic deposition of toxin into the stroma and lumen of the gland with little effect on the capsule. After injection, the muscle, subcutaneous tissues, and skin were temporarily closed with a suitable suture for transport of the animal to the necropsy facility.

Euthanasia and dissection procedures. At the necropsy facility, the animals were weighed, euthanized (approximately 118 mg/kg pentobarbital and 15 mg/kg phenytoin intravenously; Beuthanasia-D Special, Schering-Plough Animal Health Corporation, Union, NJ), and exsanguinated. The body cavity around the area of the prostate was opened and the immediate area observed for methylene blue leakage from the injection site. The prostate gland then was removed and weighed, and cross-sectional slices (length-wise, anterior to posterior) of the prostate were taken and evaluated for the distribution of methylene blue. Dye amounts in tissue were not quantified; the lost dye we discovered in animal MF2626M was by gross examination and appeared to be concentrated just ventral to the gland within the connective tissue and seminal vesicles. Tissues remaining after the study were discarded.

Results

Surgical procedure. The perineal approach provided rapid access to the prostate. An entire dosing session from the 1st incision to injection to wound closure was conducted within approximately 15 min. Blunt dissection was approached with caution, as the 2 most immediate concerns were accidental tearing of the rectum and urethra (immediately above and below the area of dissection, respectively). In studies[5] conducted subsequent to the development of this procedure, we delivered a test compound to 16 animals with this technique with no observation of incontinence (fecal or urinary), infection of the perineal tissues, or excessive discomfort to the animals 2 wk after the procedure. Two of the 19 animals undergoing this procedure suffered rectal tears during the dissection. These were repaired easily with monofilament absorbable suture. The rectal trauma was due to dissection too close to the rectal wall, and subsequent surgeries revealed no tears when applying ventral pressure during blunt dissection.

Methylene blue injections. Body weights, prostate weights, and the volumes of methlyene blue injected are given in Table 1. Animal number MF2626M received a volume of methylene blue equivalent to 96 µL per g prostate and with a needle depth of approximately 5 mm. Methylene blue flow into the seminal vesicles (Figure 2) was observed along with dye deposition towards the periphery of the gland (Figure 3), indicating that the injection volume relative to the size of the prostate and (or) needle depth was too great to retain dye within the prostate. Animal MF10263M received a volume of methylene blue equivalent to 41 µl per g prostate and again at a needle depth of approximately 5 mm. In this animal, methylene blue was

Table 1. Animal body weight, prostate weight and methylene blue volume

Animal number	Body weight (kg)	Prostate weight (g)	Total volume (µl)[a]	Unit volume (µl/g prostate)	Right prostate (µl)	Left prostate (µl)
MF2626	2.90	0.574	55	96	23	23
MF10263	4.82	1.208	50	41	25	25
MF26162	5.45	1.180	76	64	19/19[b]	19/19[b]

[a]Needle depth was approximately 5 mm for MF2626M and MF10263M and 2 to 3 mm for MF26162M.
[b]Two injections per lobe at 18.5 µl per injection.

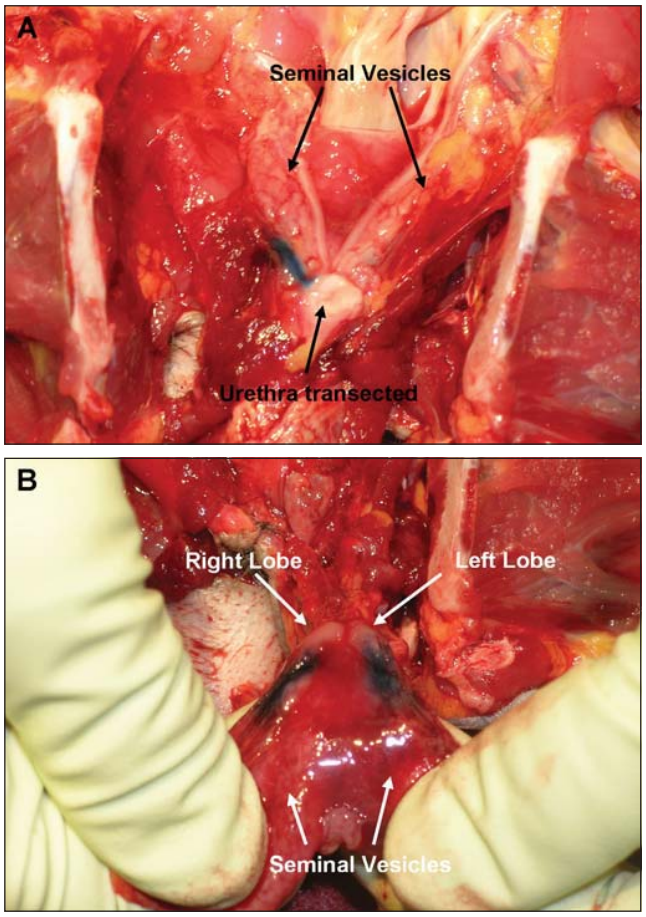

Figure 2. Photograph of methylene blue flow from the prostate to the seminal vesicles. (A) Ventral view. (B) Dorsal view.

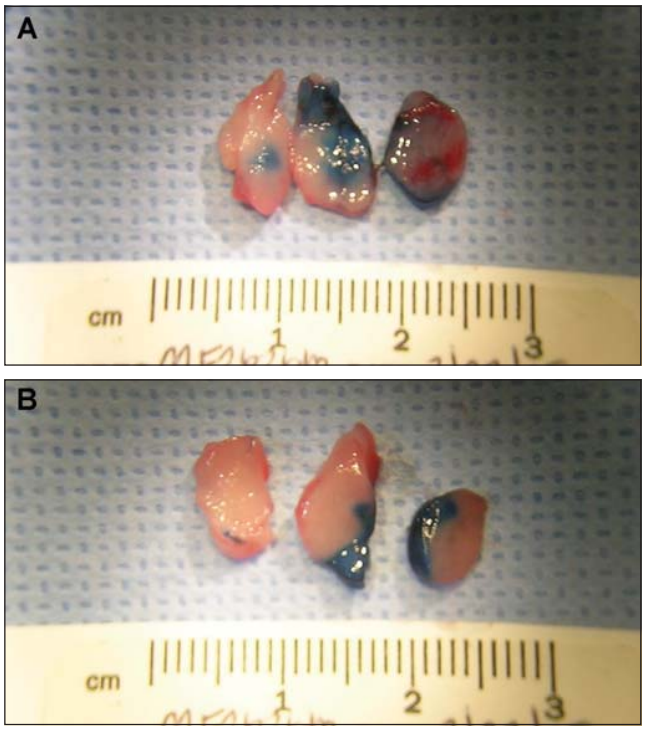

Figure 3. Photographs of cross-sectional areas of the right (A) and left (B) lobe of the prostate gland after 1 injection of methylene blue into each lobe in animal MF2626M.

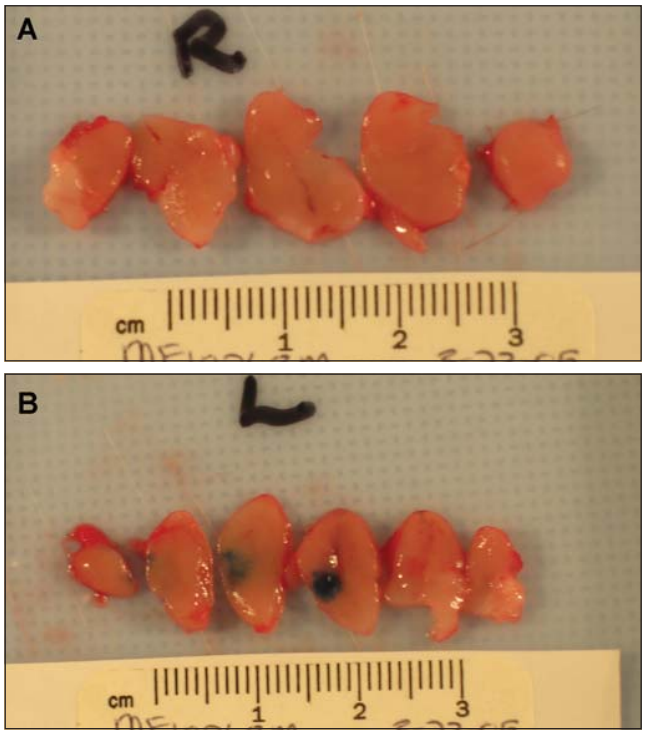

Figure 4. Photographs of cross-sectional areas of the right (A) and left (B) lobe of the prostate gland after 1 injection of methylene blue into each lobe in animal MF10263M.

deposited towards the periphery of the left lobe (Figure 4 B) but was missing in the right lobe (Figure 4 A). A needle tract in the right lobe suggests that the needle went through the gland. Dye flow into the seminal vesicles from the left lobe was not noted. Animal MF26162M received a volume of methylene blue dye equivalent to 64 µL per g prostate via 2 slow-push (approximately 30 to 45 s to minimize back pressure) injections into each lobe and to a needle depth of approximately 2 to 3 mm. Dye appeared to be more centrally and widely dispersed in the prostate of this animal relative to other animals (Figure 5). Dye flow into the seminal vesicles was not observed at this volume. The 16 perineal prostatic injection procedures performed subsequent to this study confirmed test article deposition within the treated group's prostate glands using the techniques and volumes described above for animal MF26162M.

Discussion

Radical perineal prostatectomy has been performed in people for hundreds of years and includes a procedure similar to ours in which the prostate, the fascia of Denovillier, seminal vesicles, ampullae of the vasa, and vesical neck with a portion of the trigone is exteriorized and removed for various medical indications.[4] Human literature suggests a risk of urinary and fecal incontinence after radical perineal prostatectomy. Rectal urgency was the most persistent symptom in 1 report, yet normalized in more than 90% of patients 9.5 mo postoperatively.[2] Urinary continence after radical perineal prostatectomy according to 1 study[1] was 88% and 94% at 6- and 12-mo follow-up, respectively. That study determined that to obtain complete recovery of urinary continence, in absence of detrusor instability, a functional urethral length of more than 16 mm and urethral closure pressure of more than 42 cm H_2O are needed. Because of the minimal disruption of tissues in our procedure as opposed

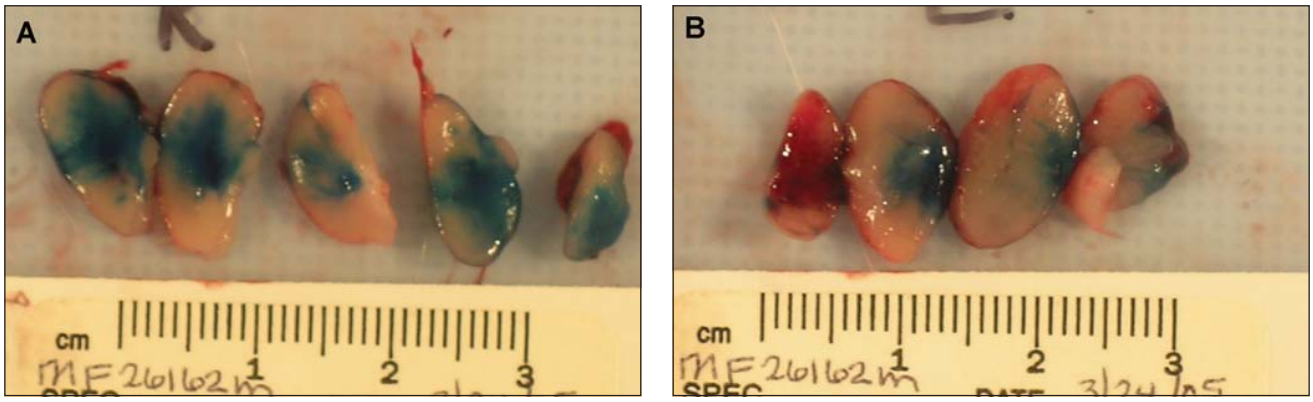

Figure 5. Photographs of cross-sectional areas of the right (A) and left (B) lobe of the prostate gland after 2 injections of methylene blue injection into each lobe in animal MF26162M.

to the human studies mentioned, it would have been unlikely to see urinary or fecal incontinence develop. In subsequent studies using this procedure, we noted only minor discomfort of the surgical site for 24 to 48 h postoperatively, as evidenced by a reluctance of some animals to sit squarely on their cage perch. Buprenorphine was given (0.02 mg/kg intramuscular; Buprenex injectable, Reckitt and Coleman Products, Richmond, VA) every 12 h for several days to control pain.

In conclusion, the perineal approach proved to be an effective means for accessing the prostate. A volume of methylene blue given up to 64 µL per g prostate was the highest volume given that did not result in a dye flow into the seminal vesicles. In addition, dividing the injection of dye into 2 injections per lobe and keeping the needle depth at 2 to 3 mm resulted in a more centrally administered and widely dispersed dye within the prostate lobe.

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