Use of a particulate extracellular matrix bioscaffold for treatment of acquired urinary incontinence in dogs

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Objective—To evaluate use of a particulate bioscaffold consisting of the extracellular matrix (ECM) of the urinary bladder from pigs for treatment of acquired urinary incontinence in dogs resistant to medical treatment.

Design—Case series.

Animals—9 female dogs with acquired urinary incontinence.

Procedure—In 6 dogs, 30 mg of particulate ECM in 1.0 mL of a carrier consisting of glycerin and saline (0.9% NaCl) solution was injected into each of 3 equally spaced sites around the circumference of the internal urethral sphincter via an endoscopic technique. In the remaining 3 dogs (control dogs), 1.0 mL of the carrier alone was injected in 3 equally spaced sites around the circumference of the internal urethral sphincter in a similar manner.

Results—For dogs treated with the ECM, median duration of urinary continence following treatment was 168 days (range, 84 to 616 days), whereas for the control dogs, median duration of urinary continence following the procedure was 14 days (range, 7 to 31 days). Two of the 3 control dogs were treated with the ECM at the end of the study and were continent for 119 and 252 days. No adverse effects were observed in any dog.

Conclusions and Clinical Relevance—Results suggest that endoscopically guided injection of particulate ECM into the internal urethral sphincter may be useful for the treatment of acquired urinary incontinence in female dogs. (*J Am Vet Med Assoc* 2005;226:1095–1097)

In dogs, acquired urinary incontinence that is not associated with infection, neurologic dysfunction, or overflow is not uncommon. The cause is not known, but there are recognized associations with early oophorectomy, female sex, and larger body size, with 78% of affected dogs weighing more than 20 kg (44 lb). Medical treatment with phenylpropanolamine (1 mg/kg [0.45 mg/lb], PO, q 12 h or q 8 h) with or without ephedrine (1.2 to 1.7 mg/kg/d [0.5 to 0.8 mg/lb/d], PO) or estrogen (1.0 mg, PO, q 24 h) is associated with a success rate of 85% to 97%.[1,2] However, some dogs may become refractory to medical treatment.

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Treatment options for dogs with acquired urinary incontinence that have become refractory to medical treatment are limited. Injection of chemically crosslinked collagen into the submucosal space of the internal urethral sphincter has been described, along with vaginal sling and colpoplasty procedures.[3-8] Injections guided by endoscopy can be readily performed in the practice setting, but the vaginal sling procedure requires open surgery that is typically performed only in selected academic institutions or specialty hospitals.

Recently, tissue-engineering methods involving a cell-based approach, a bioscaffold-based approach, a bioactive molecular approach, or some combination of these that can be used to restore structure and function to damaged or missing tissues and organs have been developed. Reconstruction of lower urinary tract tissues in humans is perhaps one of the best examples of success in this relatively new field,[9,10] and promising results have been reported following the use of bioscaffold-based tissue-engineering methods for augmentation cystoplasty, hypospadia repair, and the surgical treatment of incontinence in women.[11-16]

Allogeneic or xenogeneic **extracellular matrix (ECM)** is currently one of the more commonly used bioscaffolds for tissue-engineering procedures involving the lower urinary tract.[9-17] In particular, ECM harvested from the small intestinal submucosa or urinary bladder of pigs has shown excellent results in preclinical animal studies and clinical trials in human patients.[13,17] The ECM represents a resorbable bioscaffold material that consists of structural and functional molecules arranged in a unique 3-dimensional ultrastructure.[18] Extracellular matrix bioscaffolds that are not subjected to chemical crosslinking methods are biocompatible and are excellent substrates for host cell attachment, proliferation, and migration. These bioscaffolds typically degrade in vivo within 30 to 90 days and are replaced by site-specific host cells that repopulate and augment host tissues that are missing, injured, or otherwise deficient.[19,20]

The purpose of the study reported here was to evaluate use of a particulate bioscaffold consisting of the ECM of the urinary bladder from pigs for treatment of acquired urinary incontinence in dogs resistant to medical treatment.

Materials and Methods

Dogs—Nine female dogs with acquired urinary incontinence that were resistant to medical treatment were used in the study. Dogs were between 2.3 and 13.3 years old when acquired urinary incontinence had been diagnosed. In all dogs, the diagnosis had been made by excluding other possi-

ble causes of urinary incontinence, including infection, neoplasia, overflow incontinence, renal failure, and neurologic dysfunction. A CBC, serum biochemical profile, and urinalysis were performed in all dogs prior to entry into the study.

All dogs had received standard medical treatment for acquired urinary incontinence, including administration of phenylpropanolamine (2.2 mg/kg/d [1.0 mg/lb/d], PO), ephedrine (1.5 mg/kg/d [0.68 mg/lb/d], PO), estradiol (1 mg/d), or some combination of these. All dogs failed to respond to medical treatment or responded initially but became resistant. In all dogs, medical treatment was discontinued prior to entry into the study.

Owners of all dogs provided informed consent for enrollment of their dogs in the study and agreed to maintain a daily log of all episodes of incontinence during the posttreatment period.

Study protocol—Dogs were randomly assigned to a treatment (6 dogs) or control (3 dogs) group. Dogs in the treatment group received endoscopic injections of particulate ECM in a carrier consisting of glycerin and saline (0.9% NaCl) solution. Dogs in the control group received endoscopic injections of the carrier alone. Following treatment, dogs were monitored daily by their owners, who recorded all episodes of incontinence on log sheets provided to them by their veterinarian. Owners of the dogs were blinded to treatment group assignment.

ECM—Extracellular matrix used in the study was supplied by the manufacturer and was identical to the commercially available product.[a] Briefly, the urinary bladder was harvested from specific-pathogen-free, market-weight (ie, 110 to 120 kg [242 to 264 lb]) pigs immediately following euthanasia. The tunica serosa, tunica muscularis externa, and tunica submucosa of the urinary bladder were removed by means of mechanical delamination. The transitional epithelium was removed by use of 1.0N saline solution, which effectively lifted the transitional epithelium off the underlying basement membrane, leaving the basement membrane intact. The remaining material consisted almost exclusively of acellular ECM. Any cells in the material were removed by treatment with 0.1% peracetic acid followed by extensive rinsing in saline solution and water. The remaining ECM was acellular and retained the 3-dimensional ultrastructure that existed in vivo.

The ECM was then snap frozen in liquid nitrogen, minced into small pieces, and ground into a fine particulate. Resultant particles ranged from 50 to 150 µm in diameter. The particulate ECM was sterilized by electron beam irradiation (2.4 Mrad).

Injection procedure—For endoscopic injection, the dog was anesthetized with tiletamine-zolazepam and intubated; anesthesia was maintained with isoflurane. The dog was placed in sternal recumbency, and the perivulvar area was cleaned and prepared for cystoscopy. A 2.7-mm or 4.0-mm cystoscope was used, depending on the size of the dog. Sterile saline solution was used to expand the vaginal vault so that the urethral opening could be identified. The urethra was examined as the cystoscope was inserted to ensure that the dog did not have any anatomic abnormalities, such as ectopic ureters. Once the bladder was examined, the cystoscope was withdrawn from the bladder approximately 1.5 cm into the urethra and into the area of the internal urethral sphincter.

For dogs in the treatment group, 100 mg of particulate ECM was mixed with 2.0 mL of saline solution and 1.5 mL of glycerin, and 3.0 mL (approx 30 mg of particulate ECM/mL) of the mixture was drawn into a 3-mL syringe. A 23-gauge, 10-inch needle for injection was inserted into the working channel of the cystoscope, and the syringe containing the ECM was attached. One milliliter of the ECM suspension was then injected into the submucosal tissue at each of 3 separate locations in the area of the internal urethral sphincter (ie, at the 2, 6, and 10 o'clock positions). For each injection, the needle was tunneled in the submucosal space for a distance of approximately 1.5 cm to the location of the internal urethral sphincter to minimize backflow of material out of the needle tract. The needle was held in position for approximately 30 seconds following each injection.

For dogs in the control group, 3.0 mL of the carrier alone was injected in a similar manner. Following completion of the injections, dogs were allowed to recover from anesthesia. All dogs were discharged approximately 4 hours after treatment.

Results

All dogs in both groups were continent following treatment, according to their owners. For dogs treated with ECM, median duration of urinary continence following treatment was 168 days (range, 84 to 616 days). In contrast, durations of urinary continence for the 3 dogs in the control group were 7, 14, and 31 days. No adverse effects associated with the procedure were identified.

Two of the 3 control dogs were treated with the ECM at the end of the study and were continent for 119 and 252 days. The owner of the third control dog declined treatment with ECM.

Discussion

In the present study, duration of urinary continence for dogs treated with the particular ECM was substantially longer than duration of urinary continence for dogs treated with the carrier alone. Thus, our results suggest that endoscopically guided injection of particulate ECM into the internal urethral sphincter may be useful for the treatment of acquired urinary incontinence in dogs and that further study is warranted.

The mechanism by which ECM contributes to the remodeling of tissues, including tissues of the lower urinary tract, has been well studied.[9-11,18,21] A common feature of the remodeling that occurs following implantation of ECM in soft tissues is the infiltration of mononuclear and polymorphonuclear cells.[18,22-24] Mononuclear cells predominate after 48 to 72 hours, and the bioscaffold degrades over a 60- to 90-day period,[19,20] with the result that the implanted ECM is replaced by new host connective tissues.[16,18,24] Angiogenesis can be identified as early as 4 days after implantation of ECM and may continue for 6 to 8 weeks. It is thought that angiogenesis is a result of the release of growth factors, such as vascular endothelial growth factor and basic fibroblast growth factor, as the bioscaffold degrades.[25-27] In addition, bioactive peptides released during bioscaffold degradation have also been suggested to have angiogenic potential.[28]

Important limitations of the present study were the lack of urine pressure profilometry to verify the diagnosis of urethral sphincter incompetence prior to enrollment in the study and the inability to perform histologic evaluations of the internal urethral sphincter following treatment. However, the study of naturally occurring conditions, such as acquired urinary incontinence, in client-owned dogs often precludes the type of comprehensive diagnostic testing and follow-up that

would be desirable in a controlled study with experimental animals. At this time, there is no experimental model for acquired urinary incontinence in dogs that is resistant to medical treatment. Thus, it was not possible to perform a controlled study with experimental animals.

The procedure used in the present study required cystoscopic identification of the internal urethral sphincter and accurate injection of the material. Thus, the needs for cystoscopic equipment and adequate training in cystoscopy are potential drawbacks. On the other hand, the fact that dogs could be discharged shortly after the procedure is a potential advantage, compared with surgical methods for treatment of urinary incontinence. Additional studies incorporating larger numbers of dogs are needed to determine the time that treated animals can be expected to remain continent.

a. ACell Vet Powder, ACell Inc, Jessup, Md.

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