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Animal Models of Female Stress Urinary Incontinence

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Abstract

Purpose—Urinary incontinence affects 40% of women in the United States and stress urinary incontinence accounts for a large portion of affected patients. As defined by the International Continence Society, stress urinary incontinence is the involuntary leakage of urine upon effort, exertion, sneezing or coughing. Since the ultimate success of long-term management for any condition is based on an understanding of its pathophysiology, and because the pathophysiology of stress urinary incontinence is incompletely defined, animal models have recently been developed to better understand stress urinary incontinence and develop novel treatment alternatives.

Materials and Methods—Several animal models for urethral dysfunction have emerged in the last few years, including those based on pathophysiological theories of urethral sphincter dysfunction that were designed to simulate maternal birth trauma. Other models have focused on the creation of a durable model of dysfunction for investigating novel treatments.

Results—Since animals cannot express intent, these animal models have focused on measuring decreased urethral resistance. The most widely used methods are the sneeze test, the tilt table technique and the leak point pressure test. Newer techniques include abdominal leak point pressure, urethral pressure measurement and retrograde urethral perfusion pressure. In addition to the advantages and disadvantages of each technique, all methods measure the composite contribution to urethral resistance from smooth and striated muscle, urethral closure and connective tissue, although none measures intent.

Conclusions—We critically reviewed the different models of stress urinary incontinence and urethral dysfunction as well as the different methods of measuring urethral resistance.

Keywords

urethra; urinary incontinence; stress; female; urodynamics; models; animal

Urinary incontinence affects 40% of women in the United States. SUI, the involuntary leakage of urine upon effort, exertion, sneezing or coughing, accounts for a large portion of affected patients. More than 165,000 surgical procedures are performed for SUI in the United States. It is estimated that a third of the procedures that are performed to treat SUI are done in patients with recurrent disease. These currently performed procedures for SUI are based on compensatory and nonphysiological mechanisms. Since the ultimate success of the long-term management of any condition is based on an understanding of its pathophysiology, and because

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the pathophysiology of SUI is incompletely defined, we must invest in translational research to understand the condition and develop pathophysiologically based treatment alternatives.

Neither the spectrum of SUI nor its clinical presentations can be explained by a single risk factor or a single theory. Patients often do not become symptomatic until years after the initial trauma of childbirth, which sets up a cascade of events that continues with aging. Therefore, an opportunity exists to develop interventions aimed at preventing or slowing this cascade of events. However, to our knowledge the mechanisms involved in these events are unknown. The development of animal models is essential to understanding these events and developing preventive interventions.

To address the mechanism of SUI development one could potentially rely on epidemiological or clinical studies. However, the inherent limitations of clinical studies do not enable the investigation of mechanistic cause-and-effect relationships under controlled conditions. Since the creation of circumstances for studying the association between SUI and vaginal delivery requires an in vivo condition, an animal model that shares the biological characteristics of the human condition is the only workable alternative for investigating cause-and-effect relationships in the development of SUI as well as for preclinically testing novel treatments and prevention protocols.

ASSESSMENT OF SUI IN ANIMAL MODELS

Urinary incontinence involves the unintentional leakage of urine. However, animals cannot communicate intent. Therefore, in research involving animal models a functional surrogate, such as urethral resistance to leakage, must be used instead of knowledgeable intentional acts. Several methods of determining whether urethral resistance is decreased, which mimic the variety of clinical urodynamic tests, have been developed for use in animal, primarily rodent, models of SUI.[1–7]

Lin[1] and Sievert[2] et al developed a sneeze test for use in rodent models of SUI. In this test a whisker cut from anesthetized female rats is used to tickle the nose of the animal. Even under anesthesia the rat responds with a small sneeze, which transiently increases abdominal pressure on the bladder and lower urinary tract. In the absence of bladder pressure measurement this test provides a dichotomous result. If the animal leaks during this sneeze, she is incontinent and, if not, she is continent.[1] Uninjured control animals never leak during the sneeze test and approximately 30% that have undergone childbirth injury leak after 4 weeks.[1]

The sneeze test has been used to investigate the mechanism of reflexes involved in the continence response to sneezing. Kamo et al reported that opening the abdomen significantly decreases bladder pressure during a sneeze, indicating that the abdominal musculature contributes to the pressure increase during a sneeze.[8] The middle urethra generates a high pressure response to a sneeze that commences before the increase in abdominal pressure and is maintained relatively long after bladder pressure has returned to baseline, suggesting a sneeze induced active reflex muscle contraction to maintain continence activated directly by sneezing, rather than by afferent pathways from the bladder. By selectively transecting nerves Kamo et al determined that the reflex involved in maintaining continence during sneeze generation in urethane anesthetized female rats involves the pudendal nerve, and the nerves to the iliococcygeus and pubococcygeus muscles but not the visceral branches of the pelvic nerve or the hypogastric nerve.

Kaiho et al recently noted that intrathecal phentolamine and prazosin decreased the middle urethral response to sneezing by 11% to 15% without affecting baseline urethral pressure.[9] Intravenous nisoxetine increased the middle urethral response to sneezing by almost 20%, an increase that was eliminated by intrathecal phentolamine or prazosin. This suggests there are

at least 2 urethral continence reflex mechanisms involving noradrenergic pathways. One enhances the urethral pressure response to sneezing via α_1-adrenoceptors in the spinal cord and the other augments urethral baseline pressure via peripheral α_1-adrenoceptors.[9]

We have developed and validated a model of testing LPP in rats that mimics the Credé maneuver and can be used to statistically compare lower urinary tract pressure between experimental groups.[3,4] Urethane intraperitoneal is usually used for anesthesia because it does not mask voiding reflexes.[3] However, ketamine and xylazine intraperitoneal have also been used when repeat measurements are needed.[10] Usually the bladder is filled and bladder pressure is measured via a previously placed suprapubic bladder catheter.[3,4] Abdominal pressure is slowly increased by gentle pressure from 2 fingers placed on the closed abdomen directly over the bladder. As soon as a leak is observed at the urethral meatus, the fingers are rapidly raised and bladder pressure rapidly returns to baseline.[3,4] Increased abdominal pressure to leakage in the absence of voiding is LPP, which is approximately 40 cm H_2O in normal female rats.[11,12]

LPP can trigger a void, although it does not do so every time. Voids can be easily distinguished from leaks because the bladder pressure increase lasts longer and the volume loss is greater during voiding.[4] Despite the possibility of variability in this manually controlled technique LPP provides highly consistent results in the hands of experienced investigators.[4] Because of its ease of use and sensitivity to injury and treatments, this method of testing urethral resistance has been adopted by several groups for evaluating urethral resistance in rodent models of simulated birth injury.[13–16] A modified LPP test using a urethral instead of a suprapubic catheter has been developed.[2,10,17] However, since urethral catheters can increase bladder pressure due to increased outlet resistance,[18] we recommend using a suprapubic catheter for LPP when possible.

Another method used to determine urethral resistance in rodents raises and lowers a saline reservoir connected to a suprapubic catheter to slowly increase bladder pressure in controlled fashion.[5,6,19] The rat is mounted on a vertical tilt table to best match the orientation of the human pelvis with respect to gravity. The spinal cord of the rat is transected at the T8–T9 level to eliminate spinobulbospinal pathways, while maintaining urethral closure mechanisms intact.[5] This tilt table technique provides urethral resistance measurements similar to those of the LPP methods described[20] with similar reliability and repeatability. However, the tilt table test is more technically challenging than the LPP test because of the spinal cord transection. As a result, some groups have adopted this technique without spinal cord transection.[21]

Additional methods of measuring urethral resistance are urethral closure pressure[22,23] as well as the measurement of abdominal leak point pressure via a catheter in the rectum and measurement of RUPP via a catheter introduced into the urethral meatus.[7] Because of the distensibility of the rodent abdomen, abdominal leak point pressure is not likely to have the same degree of sensitivity to injury and dysfunction as LPP. Phull et al experimentally validated RUPP by testing rats that underwent pudendal nerve transection or urethrolysis with RUPP and LPP.[10] They found similar results in various situations, including injured/not injured and treated/not treated. When further validated, RUPP may prove to be advantageous since it does not require bladder instrumentation.

MODELS SIMULATING

MATERNAL CHILDBIRTH INJURIES

VD—Common to all investigations in this model is the use of a Foley catheter that has been modified by cutting off the tip. However, the catheter size, volume infused in the balloon and duration of vaginal distention have varied in the last 10 years. Catheter size depends largely

on rat age, weight and breeding status. Lin et al used a 5 ml 12 Foley catheter inflated with 2.0 ml water in virgin rats and 2.5 ml in retired breeder rats for 4 hours.[1] Sievert et al used a larger 22Fr catheter with a 5 ml balloon inflated for 3 hours in immediately postpartum rats.[2] They also placed the rats prone with the symphysis at the edge of the table, allowing the catheter and an attached 130 gm weight to hang freely without touching the table to simulate the forces during the second stage of delivery. Resplande et al used the same technique of vaginal distention in immediately postpartum rats using an 18Fr catheter inflated to 5 ml for 4 hours.[22] Cannon et al performed predilation of the vagina by inserting increasing sizes (24Fr to 32Fr) of urethral dilators to accommodate the vagina of virgin rats, followed by VD using 10Fr catheters inflated to 3 ml.[24]

Functionally VD results in decreased urethral resistance, as reflected by a reproducible decrease in LPP[11,12] or modified LPP[2,22] measurement. LPP decreases to approximately 60% of control values 4 days after simulated birth injury (VD),[11] demonstrating sufficient sensitivity. When incontinence was determined by a positive sneeze test performed 4 weeks following VD, a third of the rats were incontinent in the experiments of Lin et al.[1] Sievert et al found that none of the virgin rats were incontinent using the sneeze test and 29% were incontinent following delivery.[2]

Several investigators have modified the VD model of birth injury to add OVX to simulate menopause or decrease estrogen.[2,22] In the absence of VD OVX did not impact incontinence rates, although when VD was done immediately after delivery, OVX increased the incontinence rate.[2] In contrast, OVX before VD in the absence of delivery decreased the difference in LPP between the sham operated and VD groups.[25] Kuo determined that OVX does not significantly affect LPP after a single VD.[16] However, when combined with multiple vaginal traumas, a significant decrease in LPP occurred. All of these studies suggest that, if the injury is severe enough, later hormone deficiency may significantly decrease continence.

In a sequence of experiments to delineate the time course of the functional changes following VD Pan et al observed that LPP decreased to a nadir 4 days following VD and this decrease was independent of the duration of VD (1 vs 4 hours).[12] They noted that the duration of VD affected the recovery of urethral resistance. Ten days following VD LPP was significantly decreased in the 4-hour VD group but not in the 1-hour group, suggesting that longer recovery time is needed with a longer duration of VD. LPP was comparable to sham VD values in the 2 VD groups 6 weeks after the intervention, indicating functional recovery at that time. In addition, they have previously reported that a lesser duration of VD or intermittent cycling VD does not result in changes in urethral resistance.[24]

Active middle urethra sneeze induced reflex responses are present but decreased by approximately 50% after simulated childbirth injury (VD) in rats.[26] However, they were increased by intravenous nisoxetine and decreased by intrathecal phentolamine and prazosin, suggesting that this injury simulates clinical SUI and it may be treatable with a norepinephrine reuptake inhibitor.[9]

VD in rats has been shown to result in injury to the urethra, bladder, vagina and levator muscles,[1,2,11] involving smooth muscle, striated muscle and nerve fascicles.[1,11,24] Ischemia and hypoxia have also been observed[27] as well as chemokine and neuronal factors.[17,28] Lin et al noted marked cellular swelling and interstitial edema in the levator muscles 24 hours following VD.[1] After 4 weeks muscle necrosis and degeneration, an irregular shape and size of muscle fibers, and a change in the type I-to-II ratio of muscle fibers were prominent.[29] Urethral smooth muscle was decreased and disrupted and the septum between the vagina and the urethra was thinned in incontinent rats by sneeze test criteria.[1] Animals with a normal-appearing urethra were continent despite moderate or severe degrees of levator muscle changes.[1] Cannon et al

documented similar changes in the urethra following 1-hour VD, consisting of extensive disruption of the skeletal muscle layer and marked thinning of the smooth muscle fibers.[24]

In addition to muscular injury, VD results in neurogenic injury. Lin et al noted c-Fos immunoreactivity in neurons in the dorsal horn and around the central canal in spinal segments L6 and S1, indicative of nerve injury or irritation in the VD area.[1] There was also evidence of a significant loss of ganglion cells in the neural plexus posterolateral to the vagina. A marked decrease in terminal neurofilaments in the urethra of mice exposed to VD has been reported.[30] Damaser[11] and Kane[31] et al observed neurodegeneration in nerve fascicles near the EUS following VD, suggesting that neuroregenerative treatments could have a positive impact.

We have also noted that blood flow to the urethra and vagina is significantly decreased just before the release of 1-hour VD, followed by a tripling immediately after the release of VD, suggestive of ischemia/reperfusion injury.[27] Immunohistochemical data demonstrated focal hypoxia of the urothelium as well as of the submucosa, smooth muscle and potentially the EUS. Hypoxia was also observed in the bladder and vagina. However, up-regulation of the hypoxia specific chemokine stromal-derived factor 1 was not demonstrated in the pelvic organs immediately following VD.[28] However, there is 20-fold over expression of macrophage chemoattractant protein 3 in the urethra immediately following VD, which has been postulated to be secondary to a shear effect on the pelvic muscles.[28]

Due to the functional evidence of recovery VD in rats is an appropriate model in which to investigate the pathophysiology of SUI and the molecular factors that contribute to functional recovery, including stem cell mobilization following injury. Recently we noted that stem cells home to the urethra when injected intravenously after VD, a finding that is associated with accelerated recovery of urethral resistance.[32] This suggests that promoting stem cell mobilization may promote healing after delivery and have a positive effect on SUI.

Therefore, the VD model can best be used to study mechanisms of injury and tissue recovery as well as the pathophysiology of SUI. It allows the evaluation of factors, ie chemokines, neuroregenerative agents and pharmacological agents, that accelerate functional recovery[28, 31,32] as well as evaluation of the impact of contributing/decompensating factors in the pathophysiology and recovery of continence, ie diabetes, aging and obesity.

PNC—The pudendal nerve, which innervates the EUS, courses through Alcock's canal and is particularly vulnerable to stretch and crush injury during childbirth.[33] Pudendal nerve terminal motor latency, that is the time from electrical stimulation of the pudendal nerve to the initial EUS muscle response,[34] increases when the number of large myelinated motor nerve fibers decreases due to nerve damage.[33] Increased pudendal nerve terminal motor latency has positively correlated with vaginal delivery,[35] advanced age[36] and SUI.[37] Recent studies with quantitative EMG also demonstrated evidence of pelvic floor nerve injury after vaginal delivery even in the absence of incontinence symptoms.[38]

Because of this clinical evidence of pudendal nerve injury more proximal than the distal injuries incurred by vaginal delivery, we have developed an animal model focused specifically on the maternal pudendal nerve injuries incurred during childbirth.[11,39] In this model the pudendal nerve is crushed bilaterally in the ischiorectal fossa proximal to where it innervates the external urethral sphincter. Four days after bilateral PNC LPP attains a nadir and is decreased by a third, representing a significant difference from that in control animals.[11,39] Two weeks after PNC LPP returns almost to normal values but histologically the EUS and nearby nerve fascicles have not yet normalized.[39] The functional recovery of LPP at this time point is due in part to neuroregeneration, as demonstrated by a significant decrease in LPP following nerve transection 2 weeks after PNC.[40] Three months after PNC only approximately 50% of the

nerves had reinnervated the EUS.[41] Estrogen is a strong neuroregenerative agent and it facilitates accelerated recovery from PNC.[31,42]

Thus, this model is best used to study mechanisms of injury and tissue recovery in response to nerve injury. It allows evaluation of the role of neuroregenerative agents[31,42,43] in tissue recovery and the impact of contributing/decompensating factors in the pathophysiology and recovery of continence, ie diabetes, aging and obesity.

Combinational Models

The models of simulated childbirth injury can be used in combination with each other or with other animal models to investigate the interaction between different risk factors for SUI. To this end we recently began to combine PNC with VD to create a combinational model that perhaps best represents the injuries incurred due to maternal vaginal delivery. Neurotrophins, particularly brain derived neurotrophic factor, must be up-regulated by the target muscle for the regeneration of peripheral nerves after nerve injury. However, brain derived neurotrophic factor is up-regulated noticeably less in the EUS after PNC plus VD than after PNC alone,[43] suggesting that neurotrophic factors could be studied as potential methods of promoting pelvic floor reinnervation after birth injuries.

In a recent study we examined the combined effects of diabetes and birth trauma in relation to SUI.[44] In a rat model of streptozotocin induced diabetes we induced simulated childbirth injuries using VD and noted that diabetes, which is an independent risk factor for urinary incontinence, increased the severity of SUI beyond that caused by VD alone. Diabetes also delayed recovery from the simulated birth trauma and increased the histological effects of VD.[44] To our knowledge these findings provide the first line of evidence toward a possible link between vaginal delivery, SUI and diabetes in females, and it enables preclinical testing of measures to treat and prevent SUI in patients with diabetes.

MODELS OF DURABLE URETHRAL DYSFUNCTION

Urethrolysis

Urethrolysis has been used as a model of durable urethral dysfunction to create longer lasting dysfunction for testing potential treatments. After the proximal urethra is circumferentially detached by incising the endopelvic fascia the remaining urethra is then detached from the anterior vagina and pubic bone. Rodriguez et al reported that by 1 week after urethrolysis abdominal leak point pressure and RUPP decreased to approximately 50% of preoperative values and they were maintained at these levels for 24 weeks.[7] The ratio of muscle to connective tissue significantly decreased within week 1 and increased 12 weeks later. Denervation without recovery and an increase in the number of apoptotic cells 4 to 24 weeks after urethrolysis were also described. Therefore, this model creates a decrease in urethral resistance of longer duration and with more significant tissue damage than the models of simulated childbirth. Therefore, it is best used to investigate treatment interventions, ie slings and injectables, including stem cell injections, that require a durable injury model, rather than one that simulates birth injury.

Cauterization

Similar to results in the urethrolysis model, electrocauterization of the urethra of female rats produces a decrease in urethral resistance using the tilt table technique to about 50% of that in sham operated animals, which was maintained for 16 weeks.[45] Histological studies demonstrated disruption of the EUS and denervation 16 weeks after cauterization.[45] As with urethrolysis, this model creates a longer lasting decrease in urethral resistance and more significant tissue damage than childbirth simulations. It can best be used to investigate treatment interventions.

Pudendal Nerve Transection

Six weeks after injury Peng et al found a significant decrease in LPP in rats with unilateral pudendal nerve transection and a further decrease in those with bilateral nerve transection.[15] Voiding efficiency in bilaterally transected rats during cystometry was significantly decreased, while residual volume was significantly increased compared to those in unilateral and sham injured rats. Likewise unilaterally transected animals had voiding efficiency and residual volume between that of bilaterally transected and sham operated rats, and they were significantly different from each. This suggests that in female rats the pudendal nerve and its target organ, the EUS, are important not only for maintaining urethral resistance and continence during filling, but also for complete bladder emptying during voiding.

Interestingly large amplitude EUS EMG activity was recorded 6 months after unilateral or bilateral pudendal nerve transection.[15] Striated muscle bundle diameters in bilaterally transected rats were significantly decreased compared to that in unilaterally and sham injured rats, indicating that the pudendal nerve was unlikely to have regenerated after transection. Therefore, the remaining extrapudendal innervation, as indicated by EUS EMG, may be carried by the nerves to the iliococcygeus and pubococcygeus muscles, as suggested by Kamo et al.[8] This innervation may enter the urethra more distal than the pudendal nerve, as suggested by a recent anatomical study by Kim et al in female rats.[46]

Botulinum Toxin

Takahasi et al tested the injection of 10 U/200 μl botulinum-A toxin periurethrally at the mid urethra of female rats to create a durable animal model of decreased urethral resistance.[21] A significant decrease in LPP compared to controls occurred 2 weeks after injection due to the shrinkage of smooth and striated urethral muscle. Function recovered to normal levels 6 weeks after injection, demonstrating functional recovery in this model. This model would likely be most useful for testing treatments aimed specifically at restoring the function of smooth and striated muscle since each is damaged in the absence of physical trauma to the urethra.

TREATMENT TESTING

Slings

Of the various treatment options the sling procedure has become the mainstay of surgical treatment for SUI.[47] While the sling procedure offers the highest success rate, it also results in the highest morbidity and complication rates among anti-incontinence procedures.[48] Therefore, an animal model of a rat sling procedure has been developed for use in investigative studies,[13,14,49] resulting in increased urethral resistance in the short and long term.[13,14,49] In an experiment in which the suburethral portion of the sling was cut immediately after placement LPP 6 weeks later was significantly higher than in a sham sling preparation and comparable to that in an intact sling preparation.[50] Peak micturition pressure was not significantly different among the 3 groups, indicating the absence of bladder outlet obstruction after the sling procedure.

Pharmacological Therapy

Serotonin and norepinephrine reuptake inhibitors have been tested in preclinical and clinical studies of SUI, and they have been found to be efficacious despite side effects.[9] Using the botulinum-A toxin model Takahashi et al evaluated the effect of injecting escalating does of bFGF.[21] Six weeks after botulinum-A toxin injection LPP in rats with 50 and 200 μg bFGF was significantly higher than in sham injected rats. Urethral smooth and striated muscles recovered from shrinkage due to the botulinum-A toxin injection. The bFGF injection induced marked angiogenesis in the urethral periatrophic zone, suggesting that endoscopic periurethral injection of bFGF may be an attractive therapy for SUI.

Phull et al reported that angiotensin II administration did not significantly alter RUPP and ALPP in nonoperated rats.[10] However, after urethrolysis or pudendal nerve transection angiotensin II treatment resulted in a significant increase in RUPP and LPP. This suggests that, if bladder or urethral specific formulations could be found to minimize cardiovascular side effects, angiotensin II may hold potential as a treatment option.

Stem Cells

Cannon et al performed direct injection of muscle derived progenitor cells into denervated female rat urethras.[51] Injecting muscle derived progenitor cells into the denervated sphincter significantly improved fast-twitch muscle contraction amplitude. Immunohistochemistry 2 weeks following injection revealed a large amount of new skeletal muscle fiber formation at the injection site of the urethra with minimal inflammation. In subsequent experiments allogenic MDSCs significantly improved the LPP of nerve transected animals after 1 and 4 weeks[5] and increasing doses resulted in increasing improvement.[19] Similarly 2, 4 and 6 weeks after the cauterization of periurethral tissue the mean LPP in rats that received MDSCs was significantly increased compared to that in sham injected, cauterized rats.[52] MDSCs have been seeded onto a urethral sling with positive effects,[49] suggesting that slings could be an effective delivery mechanism for these cells.

Jack et al observed the viability and incorporation of processed lipoaspirate cells into the recipient smooth muscles of the bladder and urethra of Rnu athymic rats and SCID mice.[53] Eight weeks following injection processed lipoaspirate cells demonstrated in vivo expression of α-smooth muscle actin, an early marker of smooth muscle differentiation. In a subsequent study SM-hASCs (human adipose stem cells differentiated into smooth muscle) were injected into the proximal urethra of urethrolysed nude rats using engineered PLGA-ms as a carrier matrix. Only the SM-hASC plus PLGA-ms group demonstrated long-term improvement in LPP and RUPP, suggesting that PLGA-ms may not only provide an immediate bulking effect, but also localize cells to the urethra.[54]

HIGHER MAMMALIAN MODELS

As evident in this review, most of the animal models that have been developed are primarily in rats because of the expense and lengthy nature of experiments in higher mammals. Acquired SUI develops in 20% of spayed dogs and this occurrence is affected by the body weight and breed of the animals, and is not entirely predictable.[55] Therefore, the cost of working with such a model becomes a major limitation with limited added benefit. Nonhuman primates could potentially provide the advantage of having a similar orientation of the pelvis with respect to gravity as that of humans. This is likely to be even more key for studying pelvic organ prolapse than SUI due to the specific effects of gravity on prolapsing organs. Rats and mice have a different central nervous system control system at Onufrowicz's nucleus for voiding than humans.[56] Thus, it is possible that pharmacological treatments aimed at modifying central nervous system control of voiding at Onufrowicz's nucleus, ie duloxetine, should be tested in higher mammalian models that more closely match the human control system. Therefore, higher mammalian models may be useful for testing treatment interventions for SUI. However, at this time the treatments being investigated in rats, ie stem cells,[19,49,51–54] or slings,[13,14,49,50] appear to be sufficiently tested in the various rodent models.

CONCLUSIONS

Models of simulated birth injury are useful for investigating the pathophysiology of maternal birth injuries and the development of SUI as well as for developing and testing preventive measures and combination studies to investigate the interplay of multiple risk factors. Durable animal models are useful for establishing a long lasting decrease in urethral resistance for

testing treatments aimed at reversing SUI symptoms (see Appendix). The continued contributions of investigators to all of these methodologies signal increased scientific activity in investigational studies of SUI as well as a continued commitment to improving the scientific prevention of and treatment for this common condition.

Advances in science in the last 20 years now offer significant advantages in the use of the mouse as a model for many diseases, including complex conditions with genetic and environmental components, such as SUI. These advances include a fully sequenced genome, versatile strategies for making genetically modified animals, such as transgenics, knockouts, conditional or tissue specific knockouts and knock-in mutants, and the availability of probes for gene expression profiling. To capitalize on these advantages we have begun to extend our methods to the mouse.[30] Several mouse models of pelvic organ prolapse have recently been developed in mice that are missing 1 of several enzymes responsible for elastin homeostasis.[57–60] Since pelvic organ prolapse and SUI often occur together, we expect that the investigation of SUI in these mice will provide a fruitful avenue of research into the pathophysiology of how pelvic floor disorders interrelate. We expect that with the expansion of these models of SUI to mice coupling surgical and physiological studies with genetic ones will result in synergistic results as well as novel treatment and prevention options in women with SUI.

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Abbreviations and Acronyms

bFGF
basic fibroblast growth factor

EMG
Electromyography

EUS
external urethral sphincter

LPP
leak point pressure

MDSC
muscle derived stem cell

OVX
Ovariectomy

PLGA-ms
poly-lactic-glycolic acid microspheres

PNC
pudendal nerve crush

RUPP
retrograde urethral pressure profile

SUI
stress urinary incontinence

VD

vaginal distention

References

1. Lin AS, Carrier S, Morgan DM, Lue TF. Effect of simulated birth trauma on the urinary continence mechanism in the rat. Urology 1998;52:143. [PubMed: 9671889]
2. Sievert KD, Emre Bakircioglu M, Tsai T, Dahms SE, Nunes L, Lue TF. The effect of simulated birth trauma and/or ovariectomy on rodent continence mechanism. Part I: functional and structural change. J Urol 2001;166:311. [PubMed: 11435892]
3. Cannon TW, Damaser MS. Effects of anesthesia on cystometry and leak point pressure of the female rat. Life Sci 2001;69:1193. [PubMed: 11508351]
4. Damaser MS, Kim FJ, Minetti GM. Methods of testing urethral resistance in the female rat. Adv Exp Med Biol 2003;539:831. [PubMed: 15176326]
5. Lee JY, Cannon TW, Pruchnic R, Fraser MO, Huard J, Chancellor MB. The effects of periurethral muscle-derived stem cell injection on leak point pressure in a rat model of stress urinary incontinence. Int Urogynecol J Pelvic Floor Dysfunct 2003;14:31. [PubMed: 12601514]
6. Kamo I, Cannon TW, Conway DA, Torimoto K, Chancellor MB, de Groat WC, et al. The role of bladder-to-urethral reflexes in urinary continence mechanisms in rats. Am J Physiol Renal Physiol 2004;287:F434. [PubMed: 15113743]
7. Rodriguez LV, Chen S, Jack GS, de Almeida F, Lee KW, Zhang R. New objective measures to quantify stress urinary incontinence in a novel durable animal model of intrinsic sphincter deficiency. Am J Physiol Regulat Integr Comp Physiol Regulat Integr Comp Physiol 2005;288:R1332.
8. Kamo I, Torimoto K, Chancellor MB, de Groat WC, Yoshimura N. Urethral closure mechanisms under sneeze-induced stress condition in rats: a new animal model for evaluation of stress urinary incontinence. Am J Physiol Regulat Integr Comp Physiol 2003;285:R356.
9. Kaiho Y, Kamo I, Chancellor MB, Arai Y, de Groat WC, Yoshimura N. Role of noradrenergic pathways in sneeze-induced urethral continence reflex in rats. Am J Physiol Renal Physiol 2007;292:F639. [PubMed: 17047168]
10. Phull H, Salkini M, Escobar C, Purves T, Comiter CV. The role of angiotensin II in stress urinary incontinence: a rat model. Neurourol Urodyn 2007;26:81. [PubMed: 17029249]
11. Damaser MS, Broxton-King C, Ferguson C, Kim FJ, Kerns JM. Functional and neuroanatomical effects of vaginal distention and pudendal nerve crush in the female rat. J Urol 2003;170:1027. [PubMed: 12913764]
12. Pan HQ, Kerns JM, Lin DL, Liu S, Esparza N, Damaser MS. Increased duration of simulated childbirth injuries results in increased time to recovery. Am J Physiol Regul Integr Comp Physiol 2007;292:R1738. [PubMed: 17204590]
13. Hijaz A, Daneshgari F, Cannon T, Damaser M. Efficacy of a vaginal sling procedure in a rat model of stress urinary incontinence. J Urol 2004;172:2065. [PubMed: 15540790]
14. Hijaz A, Bena J, Daneshgari F. Long-term efficacy of a vaginal sling procedure in a rat model of stress urinary incontinence. J Urol 2005;173:1817. [PubMed: 15821594]
15. Peng CW, Chen JJ, Chang HY, de Groat WC, Cheng CL. External urethral sphincter activity in a rat model of pudendal nerve injury. Neurourol Urodyn 2006;25:388. [PubMed: 16637068]
16. Kuo HC. Effects of vaginal trauma and oophorectomy on the continence mechanism in rats. Urol Int 2002;69:36. [PubMed: 12119437]
17. Sievert KD, Bakircioglu ME, Tsai T, Nunes L, Lue TF. The effect of labor and/or ovariectomy on rodent continence mechanism—the neuronal changes. World J Urol 2004;22:244. [PubMed: 15365750]
18. Scaldazza CV, Morosetti C. Effect of different sized transurethral catheters on pressure-flow studies in women with lower urinary tract symptoms. Urol Int 2005;75:21. [PubMed: 16037703]
19. Kwon D, Kim Y, Pruchnic R, Jankowski R, Usiene I, de Miguel F, et al. Periurethral cellular injection: comparison of muscle-derived progenitor cells and fibroblasts with regard to efficacy and tissue contractility in an animal model of stress urinary incontinence. Urology 2006;68:449. [PubMed: 16904482]

20. Conway DA, Kamo I, Yoshimura N, Chancellor MB, Cannon TW. Comparison of leak point pressure methods in an animal model of stress urinary incontinence. Int Urogynecol J Pelvic Floor Dysfunct 2005;16:359. [PubMed: 16132162]
21. Takahashi S, Chen Q, Ogushi T, Fujimura T, Kumagai J, Matsumoto S, et al. Periurethral injection of sustained release basic fibroblast growth factor improves sphincteric contractility of the rat urethra denervated by botulinum-A toxin. J Urol 2006;176:819. [PubMed: 16813954]
22. Resplande J, Gholami SS, Graziottin TM, Rogers R, Lin CS, Leng W, et al. Long-term effect of ovariectomy and simulated birth trauma on the lower urinary tract of female rats. J Urol 2002;168:323. [PubMed: 12050564]
23. Bae JH, Moon DG, Lee JG. The effects of a selective noradrenaline reuptake inhibitor on the urethra: an in vitro and in vivo study. BJU Int 2001;88:771. [PubMed: 11890252]
24. Cannon TW, Wojcik EM, Ferguson CL, Saraga S, Thomas C, Damaser MS. Effects of vaginal distension on urethral anatomy and function. BJU Int 2002;90:403. [PubMed: 12175397]
25. Ferguson CL, Lin DL, Rao S, Damaser MS. Short-term functional and neuroregenerative response of the urethra to ovariectomy and vaginal distension in female rats. Int Urogynecol J Pelvic Floor Dysfunct 2005;16:119. [PubMed: 15789145]
26. Kamo I, Kaiho Y, Canon TW, Chancellor MB, de Groat WC, Prantil RL, et al. Functional analysis of active urethral closure mechanisms under sneeze induced stress condition in a rat model of birth trauma. J Urol 2006;176:2711. [PubMed: 17085201]
27. Damaser MS, Whitbeck C, Chichester P, Levin RM. Effect of vaginal distension on blood flow and hypoxia of urogenital organs of the female rat. J Appl Physiol 2005;98:1884. [PubMed: 15591288]
28. Woo LL, Hijaz A, Kuang M, Penn MS, Damaser MS, Rackley RR. Over expression of stem cell homing cytokines in urogenital organs following vaginal distention. J Urol 2007;177:1568. [PubMed: 17382780]
29. Bakircioglu ME, Sievert KD, Lau A, Lin CS, Lue TF. The effect of pregnancy and delivery on the function and ultra-structure of the rat bladder and urethra. BJU Int 2000;85:350. [PubMed: 10671896]
30. Lin YH, Liu G, Daneshgari F. A mouse model of simulated birth trauma induced stress urinary incontinence. Neurourol Urodyn. 2007Epub ahead of print
31. Kane DD, Kerns JM, Lin DL, Damaser MS. Early structural effects of oestrogen on pudendal nerve regeneration in the rat. BJU Int 2004;93:870. [PubMed: 15050008]
32. Woo LL, Hijaz A, Mei K, Mal N, Penn MS, Rackley RR, et al. Stem cell homing and functional recovery after simulated childbirth injury in female rats. J Urol 2007;177:438.abstract 1330
33. Snooks SJ, Swash M, Henry MM, Setchell M. Risk factors in childbirth causing damage to the pelvic floor innervation. Int J Colorectal Dis 1986;1:20. [PubMed: 3598309]
34. Benson JT. Neurophysiology of the female pelvic floor. Curr Opin Obstet Gynecol 1994;6:320. [PubMed: 7742493]
35. Tetzschner T, Sorensen M, Jonsson L, Lose G, Christiansen J. Delivery and pudendal nerve function. Acta Obstet Gynecol Scand 1997;76:324. [PubMed: 9174425]
36. Olsen AL, Ross M, Stansfield RB, Kreiter C. Pelvic floor nerve conduction studies: establishing clinically relevant normative data. Am J Obstet Gynecol 2003;189:1114. [PubMed: 14586364]
37. Snooks SJ, Barnes PR, Swash M. Damage to the innervation of the voluntary anal and periurethral sphincter musculature in incontinence: an electrophysiological study. J Neurol Neurosurg Psychiatry 1984;47:1269. [PubMed: 6512547]
38. Gregory WT, Lou JS, Stuyvesant A, Clark AL. Quantitative electromyography of the anal sphincter after uncomplicated vaginal delivery. Obstet Gynecol 2004;104:327. [PubMed: 15292007]
39. Ahn H, Lin DL, Esparza N, Damaser MS. Short-term timecourse of bilateral pudendal nerve injury on leak-point pressure in female rats. J Rehabil Res Dev 2005;42:109. [PubMed: 15742255]
40. Damaser MS, Samplaski MK, Parikh M, Lin DL, Rao S, Kerns JM. Time course of neuroanatomical and functional recovery after bilateral pudendal nerve injury in female rats. Am J Physiol Renal Physiol 2007;293:F1614. [PubMed: 17728381]
41. Kerns JM, Damaser MS, Kane JM, Sakamoto K, Benson JT, Shott S, et al. Effects of pudendal nerve injury in the female rat. Neurourol Urodyn 2000;19:53. [PubMed: 10602248]

42. Ahmed Y, Lin DL, Ferguson C, Esparza N, Damaser MS. Effect of estrogen on urethral function and nerve regeneration following pudendal nerve crush in the female rat. J Urol 2006;175:1948. [PubMed: 16600804]
43. Damaser MS, Lin DL, Kerns JM, Sypert DF, Eggers L, Pan HQ. Urodynamic function and expression of neurotrophins after a maternal childbirth injury model of vaginal distension and pudendal nerve crush in rats. Neurourol Urodyn 2007;26:696.
44. Kim JH, Huang X, Liu G, Moore C, Bena J, Damaser MS, et al. Diabetes slows the recovery from urinary incontinence due to simulated childbirth in female rats. Am J Physiol Regul Integr Comp Physiol 2007;293:R950. [PubMed: 17491107]
45. Chermansky CJ, Cannon TW, Torimoto K, Fraser MO, Yoshimura N, de Groat WC, et al. A model of intrinsic sphincteric deficiency in the rat: electrocauterization. Neurourol Urodyn 2004;23:166. [PubMed: 14983430]
46. Kim R, Kerns JM, Liu S, Nagel T, Zaszczurynski P, Lin DL, et al. Striated muscle and nerve fascicle distribution in the female rat urethral sphincter. Anat Rec (Hoboken) 2007;290:145. [PubMed: 17441207]
47. Kim HL, Gerber GS, Patel RV, Hollowell CM, Bales GT. Practice patterns in the treatment of female urinary incontinence: a postal and internet survey. Urology 2001;57:45. [PubMed: 11164141]
48. Leach GE, Dmochowski RR, Appell RA, Blaivas JG, Hadley HR, Luber KM, et al. Female Stress Urinary Incontinence Clinical Guidelines Panel summary report on surgical management of female stress urinary incontinence. The American Urological Association. J Urol 1997;158:875. [PubMed: 9258103]
49. Cannon TW, Sweeney DD, Conway DA, Kamo I, Yoshimura N, Sacks M, et al. A tissue-engineered suburethral sling in an animal model of stress urinary incontinence. BJU Int 2005;96:664. [PubMed: 16104928]
50. Hijaz A, Daneshgari F, Huang X, Bena J, Liu G, Saffore L, et al. Role of sling integrity in the restoration of leak point pressure in the rat vaginal sling model. J Urol 2005;174:771. [PubMed: 16006974]
51. Cannon TW, Lee JY, Somogyi G, Pruchnic R, Smith CP, Huard J, et al. Improved sphincter contractility after allogenic muscle-derived progenitor cell injection into the denervated rat urethra. Urology 2003;62:958. [PubMed: 14624934]
52. Chermansky CJ, Tarin T, Kwon DD, Jankowski RJ, Cannon TW, de Groat WC, et al. Intraurethral muscle-derived cell injections increase leak point pressure in a rat model of intrinsic sphincter deficiency. Urology 2004;63:780. [PubMed: 15072911]
53. Jack GS, Almeida FG, Zhang R, Alfonso ZC, Zuk PA, Rodriguez LV. Processed lipoaspirate cells for tissue engineering of the lower urinary tract: implications for the treatment of stress urinary incontinence and bladder reconstruction. J Urol 2005;174:2041. [PubMed: 16217390]
54. Zeng X, Jack G, Zhang R, Leung J, Wu B, Rodriguez LV. Fibroblats and adipose derived stem cells in the treatment of stress urinary incontinence: comparison of functional outcomes. J Urol 2007;177:554.abstract 1671
55. Arnold S. Urinary incontinence in castrated bitches. Part 1: significance, clinical aspects and etiopathogenesis. Schweiz Arch Tierheilk 1997;139:271.
56. de Groat WC, Fraser MO, Yoshiyama M, Smerin S, Tai C, Chancellor MB, et al. Neural control of the urethra. Scand J Urol Nephrol, suppl 2001;35:35. [PubMed: 11409613]
57. Liu X, Zhao Y, Gao J, Pawlyk B, Starcher B, Spencer JA, et al. Elastic fiber homeostasis requires lysyl oxidase-like 1 protein. Nat Genet 2004;36:178. [PubMed: 14745449]
58. Liu X, Zhao Y, Pawlyk B, Damaser M, Li T. Failure of elastic fiber homeostasis leads to pelvic floor disorders. Am J Pathol 2006;168:519. [PubMed: 16436666]
59. Liu G, Daneshgari F, Li M, Lin D, Lee U, Li T, et al. Bladder and urethral function in pelvic organ prolapsed lysyl oxidase like-1 knockout mice. BJU Int 2007;100:414. [PubMed: 17555473]
60. Drewes PG, Yanagisawa H, Starcher B, Hornstra I, Csiszar K, Marinis SI, et al. Pelvic organ prolapse in fibulin-5 knockout mice: pregnancy-induced changes in elastic fiber homeostasis in mouse vagina. Am J Pathol 2007;170:578. [PubMed: 17255326]

APPENDIX

Rat Models of Urethral Dysfunction

Model	Method	Advantages	Disadvantages	Translational Research Use
VD[1,2,11,12,22,24,28]	Balloon distention of the vagina	Reversible injury	Nonspecific injury	Best used in studying mechanisms of injury and tissue recovery as well as pathophysiology
		Functional recovery depends on duration of VD	Not a durable injury	
		Simulates birth trauma		
		Technique is easily done and results are reproducible		
				Allows for evaluation of factors (chemokines, neuroregenerative and medication selective serotonin reuptake inhibitor) that accelerate functional recovery[28–32]
				Allows for evaluation of the impact of contributing/ decompensating factors on pathophysiology and recovery of incontinence (diabetes,44 aging and obesity)
PNC[11,39]	Crush injury to the pudendal nerve in the ischiorectal fossa	Reversible injury	Technique requires fine surgical skills and operating microscopy	Best used in studying mechanisms of injury and tissue recovery in response to isolated nerve injury.
		Functional recovery occurs in 2 weeks		
		Selective neurogenic model	Not a durable injury	
		Can be used in combination with VD for a more profound type of injury[40]		Allows for evaluation of the role of neuroregenerative agents in tissue recovery[31, 42,43]
				Allows for evaluation of the impact of contributing/ decompensating factors on pathophysiology and recovery of incontinence (diabetes,44 aging and obesity)
Pudendal nerve transection[15,46]	Complete transection of the pudendal nerve in the ischiorectal fossa	Durable injury	Technique requires fine surgical skills and operating microscopy	Best used to investigate treatment interventions: slings, stem cell injection and different injectable material
		Selective neurogenic model		
			Not an injury that occurs naturally in childbirth	
Urethrolysis[7]	Proximal urethra is circumferentially detached by incising the endopelvic fascia and the remaining urethra is then detached from the anterior vagina and pubic bone	Durable injury	Not an injury that occurs naturally in childbirth	Best used to investigate treatment interventions: slings and injectables, including stem cell injection
		More significant damage compared to VD model		
		Technique is easily done		
Cauterization[45]	Electrocauterization of the urethra	Durable injury	Not an injury that occurs naturally in childbirth	Best used to investigate treatment interventions: slings and injectables, including stem cell injection
		More significant damage compared to VD model		
		Technique is easily done		
Botulinum toxin[21]	Periurethrally 10 U/ 200 μl at the mid urethra	Reversible model	Not an injury that occurs naturally in childbirth	Allows for evaluation of interventions that accelerate tissue recovery (fibroblast growth factor)[21]
		Recovery occurs in 6 weeks		