Effects of bilateral levator ani nerve injury on pelvic support in the female squirrel monkey

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OBJECTIVE: To determine whether experimental denervation of the levator ani (LA) and its subsequent atrophy contribute to the development of pelvic organ prolapse in the squirrel monkey.

STUDY DESIGN: Thirty-seven female monkeys were evaluated including 7 that underwent bilateral LA neurectomy (bLAN), 17 nulliparous monkeys without prolapse, 7 parous monkeys without prolapse, and 6 parous monkeys with prolapse. Magnetic resonance imaging was used to calculate LA muscle volumes and obtain measurements of the position of bladder and cervix. Repeat observations in bLAN females occurred at different times in relation to parturition.

RESULTS: LA volumes were reduced in bLAN monkeys ($P = .02$). Bladder ($P = .03$) and cervix ($P = .04$) positions varied between groups, with nulliparous females having the most cephalad positions and females with prolapse having the most caudal positions. Bladder descent was observed in a subset of 4 bLAN females that experienced vaginal parturition ($P = .04$) and correlated with external findings of vaginal prolapse.

CONCLUSION: Bilateral transection of the LA nerve results in atrophy of denervated LA muscles but not a loss of pelvic support in nulliparous monkeys, suggesting that connective tissue components compensate for weakened pelvic floor muscles. LA denervation may accelerate the onset of vaginal prolapse subsequent to parturition.

Key words: denervation, levator ani, levator ani nerve, magnetic resonance imaging, pelvic organ prolapse, squirrel monkey, vaginal delivery

Cite this article as: Pierce LM, Coates KW, Kramer LA, et al. Effects of bilateral levator ani nerve injury on pelvic support in the female squirrel monkey. Am J Obstet Gynecol 2008;198:585.e1-585.e8.

It is estimated that 1 in 9 women will undergo a surgical procedure for pelvic organ prolapse (POP) or urinary incontinence during their lifetime.[1] Although existing data reveal that POP is associated with age, parity, and ethnicity,[2,3] little is known about its etiology. Contributing factors are thought to include denervation injury and stretch-related injury to the striated pelvic floor muscles during vaginal delivery, as well as defects in the fibromuscular connective tissues of the vaginal wall and supportive structures.[4-8]

Because performing properly designed experimental studies in women is challenging due to ethical constraints, we have established the squirrel monkey as an animal model of vaginal birth-associated prolapse and have characterized pelvic floor innervation in this model.[9-15] In the current study, we used an experimental approach to determine whether denervation of the levator ani (LA) and its subsequent atrophy contribute to the development of POP in this species.

Magnetic resonance imaging (MRI) as a method for the assessment of POP in women remains investigational. However, a number of researchers have reported the use of conventional 2-dimensional images and 3-dimensional modeling techniques combined with static and dynamic measurements to compare asymptomatic subjects with those experiencing symptoms of prolapse or stress urinary incontinence.[16-19] Hoyte et al[16] were among the first to report differences in the position and shape of LA muscles in patients with POP during dynamic maneuvers (maximum Valsalva) as well as with static imaging. MRI and 3-dimensional modeling have also proven useful to compare muscle volumes in living squirrel monkeys with and without POP.[14] In the current study, we developed a method for the dynamic evaluation of the squirrel monkey pelvic floor with visualization of pelvic organ descent in a series of MR images obtained in the midsagittal plane enabling the measurement of changes in position of the pelvic viscera with applied abdominal pressure.

In this study, we used MRI in combination with a previously reported pelvic examination procedure[9,10] to assess LA

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Presented at the 28th Annual Scientific Meeting of the American Urogynecologic Society, Hollywood, FL, Sept. 27-29, 2007.

Received July 11, 2007; revised Nov. 9, 2007; accepted Dec. 5, 2007.

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This work was supported by NIH Grant HD38655, the Mary Jane Noble Foundation, the Baden Family Center, and the Scott, Sherwood and Brindley Foundation.

0002-9378/$34.00 • © 2008 Mosby, Inc. All rights reserved. • doi: 10.1016/j.ajog.2007.12.001

atrophy and the development of POP in a cohort of 7 female squirrel monkeys that underwent bilateral transection of the LA nerve. Repeat observations in these neurectomized animals occurred at different times in relation to parturition for periods of up to 6 years postneurectomy. Comparisons were made to monkeys with intact nerves, including parous females with naturally occurring POP, parous females without POP, and nulliparous females.

Materials and Methods

Animal subjects

Animals were obtained from the National Squirrel Monkey Breeding and Research Resource at the University of South Alabama (Mobile, AL) and from Buckshire Corporation (Perkasie, PA) and were housed at the Scott & White Memorial Hospital Animal Facility in Temple, TX. A total of 37 female squirrel monkeys were used, ranging in age from 2 to 25 years, including 6 parous females with naturally occurring POP, 7 parous females without POP, 17 nulliparous females without POP, and 7 females that underwent bilateral transection of the LA nerve. All females with POP had anterior and/or posterior segment defects that extended beyond the vaginal opening using a previously reported pelvic examination procedure.[9,10] Nineteen monkeys used as part of this study were also used in prior studies to examine LA muscle volume[14] and histopathology.[15] Guidelines for the care and use of these animals, approved by The Scott & White Institutional Animal Care and Use Committee, were followed.

Bilateral levator ani neurectomy (bLAN)

As previously described,[11] the abdomen and pelvis from 7 nulliparous monkeys (6 animals = 2 years old; 1 animal = 5 years old) were entered through a vertical midline abdominal incision, and approximately 1 cm segments of LA nerve were obtained bilaterally and processed for microscopy to confirm that the transected structures identified grossly were in fact nerve. Histological analysis verified nerve tissue in the harvested specimens from all 7 animals.

Four of 7 bLAN animals underwent 2 subsequent full-term pregnancies at 2 and 3 years postneurectomy, 2 died from obstetric complications during their first pregnancy at 2 years postneurectomy, and 1 remained nulliparous. LA muscles from the 2 monkeys that died were processed for histology to confirm muscle denervation.[15] Surviving parous bLAN animals were observed for a period of up to 4 years postneurectomy, and the nulliparous bLAN animal was observed for 6 years.

MRI of the pelvic floor in squirrel monkeys

MRI was performed using a validated procedure as previously described.[14] A sequence of 224 T1-weighted, gradient echo, axial images was acquired during a 4.7 min interval. After the procedure, a neonatal blood pressure cuff was placed around the animal's abdomen to provide transabdominal pressure. Two procedures were used, including a static and a dynamic series of images. The static series involved inflating the cuff with 40 cc of air and then performing a second high resolution scan while inflation pressure was maintained. The dynamic series involved applying a similar amount of inflation pressure during the scanning sequence in which serial sagittal images along the midline were obtained. During both procedures, cuff inflation produced abdominal pressure sufficient to cause animals to void or display vaginal prolapse. The image files were recorded in DICOM format and processed using 3D-Doctor software (Able Software Corporation, Lexington, MA) for review, manipulation, measurement, and analysis.

By tracing regions of interest in each axial image, 2 experienced investigators (TJK and LAK) independently measured volumes of the left and right LA and obturator internus muscles in 7 nulliparous females that underwent bLAN 2 to 3 years prior to MRI and in 7 uninjured nulliparous females with normal support matched in age and weight to injured females. In all 37 monkeys, measurements of the position of the most caudal aspect of the bladder neck (defined as the urethrovesical junction) and cervix (as a marker of uterine position) relative to a bony structure reference line were made in sagittal views. The reference line extended from the cephalad aspect of the pubic symphysis to the caudal aspect of the first coccygeal vertebra. This line is approximately parallel to the floor in animals standing upright. Positions of bladder neck and cervix were measured in either the static or dynamic series of images by 2 independent reviewers (TJK and JCB) in animals before and after applying abdominal pressure. Repeat observations by MRI and by pelvic examination[9,10] in bLAN females occurred at different times in relation to parturition. For all measurements, either 1 or both independent observers were blinded to monkey characteristics and treatment group.

Statistical analysis

Results are expressed as means with standard error (SE). Multivariate analysis of variance (ANOVA) with Wilks test or univariate ANOVA was used to assess demographic variables between groups, and Duncan's test was used for post-hoc comparisons. ANOVA was also used to assess differences in muscle volumes, positions of bladder and cervix, and perineal measurements among treatment groups. Homogeneity of variance was assessed and nonparametric comparison used, if needed. The paired t test or the Wilcoxon signed rank test was performed to compare pre- and postdelivery measurements from bLAN animals. Statistical significance was determined by $P < .05$.

Results

Muscle volumes

LA and obturator internus muscle volumes were evaluated in 14 nulliparous monkeys, including 7 with bLAN performed 2 to 3 years earlier and 7 without nerve injury (Table 1). Animals with bLAN did not differ ($P = .78$) from those without neurectomy in age and weight. Individual LA muscle volumes did differ between groups, with smaller volumes observed in the bLAN cohort (322 mm^3