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A STUDY OF ANAL CONTINENCE MECHANISMS IN A RAT MODEL: EFFECT OF PUDENDAL NERVE TRANSECTION AND SPHINCTEROTOMY.

Hypothesis / aims of study

Fecal incontinence is a major debilitating condition that primarily affects women due to childbirth injuries. Most women who have acute injury to their anal sphincters remain asymptomatic, and injuries remain occult for a long time before women present with symptoms of fecal incontinence. Although anal physiology has been investigated previously, no animal model has been established for studying the mechanism of this injury, deterioration of continence with age, as well as potential treatments. Rat anal sphincter anatomy is similar to human, which makes this animal suitable for studying the etiologic mechanisms of fecal incontinence. Our aim was to study the long term effect of pudendal nerve transection (PNT) and sphincterotomy on the anal sphincter.

Study design, materials and methods

Seventy-two virgin Sprague Dawley rats were grouped into control (C=12), sphincterotomy (SG n=30), and pudendal nerve transection (PNTG n=30) group and were evaluated at 1 and 4 days as well as 2 and 4 weeks after injury (PI). The animals were anaesthetized using a mixture of ketamine (100mg/kg) and xylazine (10mg/kg) i.p. Anorectal pressure (AP) was monitored using a saline-filled latex balloon connected through a saline filled PE-90 tubing to a pressure transducer, amplifier, and digital data recording system. After establishing balloon baseline pressure for 10-15 minutes, testing was started. The baseline resting pressures and anal contraction pressures were monitored and outcome variables were assessed. Anal sphincter electromyographic (EMG) signals were simultaneously recorded using a concentric needle placed in the anal sphincter 3-4 mm deep on the left side and connected to an amplifier and the digital data recording system.

Rats in the SG group received a sphincterotomy after initial AP and EMG recordings, inflicting a 3-4 mm incision at the 7'o-clock position in the anus. AP and EMG testing was repeated after sphincter injury. Rats in the PNTG group had pudendal nerve isolation prior to the experiment as follows: an incision was made at the sacro-coccygeal area, and the pudendal nerve was identified and meticulously isolated with a silk 3-0 suture bilaterally. The suture was arranged so it could be pulled after initial AP and EMG monitoring, thus transecting and avulsing the pudendal nerve one side at a time. AP and EMG recordings were made after unilateral as well as bilateral nerve transection. Animals were then euthanized by administration of Pentobarbital (80-100mg/kg) intra-cardiac and the anorectal area was carefully dissected, embedded in paraffin, sectioned (5μm), and stained with Masson's trichome. Histology and EMG signals were assessed qualitatively. Pressure data was analyzed in terms of resting pressure (RP), peak contraction (PC), number of peaks per contraction (NP), time to peak contraction (PT), and interval between contractions (CI). Data are presented as mean ± standard deviation of each group and were analyzed using a Wilcoxon two sample comparison test, with $p<0.05$ indicating a significant difference between groups.

Results

Baseline testing was consistent in all groups and demonstrated spontaneous and periodic AP and EMG recordings. One and 4 days after sphincterotomy there was a significant decrease in RP (7.0±0.72; 6.0±0.67 cmH_20, respectively) and PC (8.76±0.58;8.0±0.47 cmH_20, respectively) compared to control RP (8.1± 0.44;9.0±0.61 cmH20, respectively) and PC (11.9±0.60;11.2±0.86 cmH20, respectively; Figure 1). AP recovery was seen at 2 weeks and was comparable to controls at 4 weeks PI. EMG data did not show significant differences after SG compared to controls. One and 4 days after pudendal nerve transection, there was a significant decrease in RP (6.9±0.46;6.71±0.42 cmH_20, respectively) and PC (9.3±0.34;9.0±0.51 cmH20) compared to control ($p<0.001$; Figure 1). Transient recovery was seen at 2 weeks, however a significant decrease was seen 4 weeks post injury ($p=0.01$). PT, NP and CI did not show significant differences compared to controls after either sphincterotomy or pudendal nerve transection. PNTG EMG analysis showed loss of activity on day 1 and 4 post injury, however some EMG activity returned 2 and 4 weeks post injury. SG histology showed sphincter transection at day 1 day 4 with mild inflammation and fibrosis 2 weeks and increased fibrosis 4 weeks PI (Figure 2). PNTG histology was same as control on day 1 and 4, however, external anal sphincter (EAS) atrophy was seen starting at 2 weeks and marked atrophy occurred at 4 weeks.

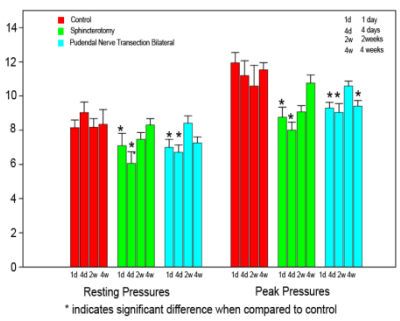

Figure 1. Resting and peak anal at different time Intervals after injury.

Fig 2 A

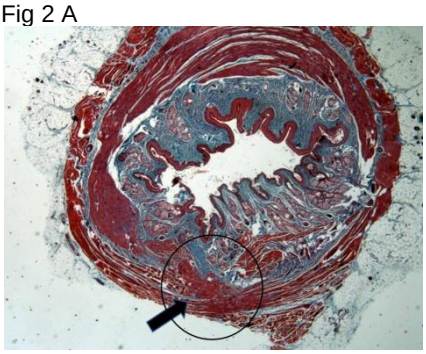

Fig 2 B

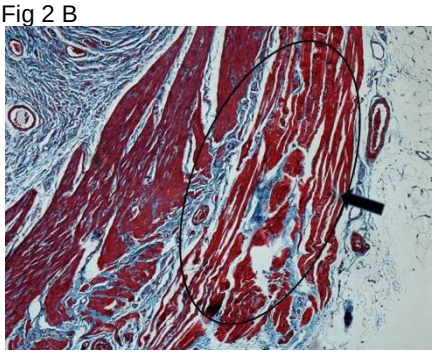

Figure 2 A and B. Transverse section showing pressures fibrosis (A-arrow) in the anal sphincter 4 weeks post injury and 2b showing EAS atrophy (B-arrow).

Interpretation of results

The low values in SG indicates significant sphincter laceration that affects the anal pressures during first few weeks of injury, the anal pressure improvement seen is an indication of gradual healing response from this trauma [1]. PNTG early decreased in anal pressure also indicates acute loss of neural control [2], transient increase of AP at 2nd week probably signifies attempted neural

recovery. However, due to the permanent nerve damage, a second wave of significant pressure decreased after 4 weeks was seen. This two different injury models (mechanical and denervation) closely simulates the childbirth trauma in women [3].

Concluding message

The female rat is a suitable and reliable model for studying the physiology of the anal sphincters and the mechanisms of fecal incontinence Pudendal nerve transection causes atrophy of the external anal sphincter and chronically alters AP. The EMG signal was significantly decreased only after pudendal nerve transection. Sphincterotomy produces a temporary injury which recovers without repair in this model. The inflammatory response post injury results in a time related recovery after several weeks.

References

1. Obstetrics and Gynecology 3(2):332-340 2008
2. Neurourology and Urodynamics 1: 53-69, 2000
3. AJ Obs Gyne 182:427-432 2001

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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Institute of Animal Care and Control (IACUC)**