

EFFECTS OF OVARIECTOMY AND HORMONE REPLACEMENT ON SUBMUCOSAL COLLAGEN AND BLOOD VESSELS OF THE ANAL CANAL OF RATS.

Hypothesis / aims of study

The contribution of mucosal or submucosal connective and vascular tissues to anal pressure in women has not been fully appreciated in clinical practice in contrast to their roles in the female urethra [1,2]. Therapeutic success of submucosal anal injection of various bulking agents in women with idiopathic fecal incontinence, however, suggests that a situation analogous to the female urethra might exist in the anal canal [3]. Each of these two connective tissue components, collagen and vascular plexuses, also appear to have some dependence on estrogen and possibly progesterone, as observed in the female urethra [2]. The presence of estrogen and progesterone receptors in the mucosal and submucosal connective tissue of the female anal canal and the ability of these hormones to induce structural changes in individual components such as collagen and blood vessels would provide evidence for an action of female sex hormones on fecal control. Our study attempts to investigate this hypothesis in adult female rats.

Study design, materials and methods

Experiments were performed on sections of the anal canal (the region extending from the beginning of the anal columns to the orifice) of ovariectomized adult Wistar female rats (weight= 200-250g, age= 5-6 months) following 28 daily subcutaneous injections of 17- β estradiol 10 μ g/kg/day (n=6, OVX + E, Group 1), medroxyprogesterone acetate 0.2 μ g/kg/day (n=6, OVX + P, Group 2), both drugs (n=6, OVX +E+P, Group 3) or vehicle (n=6, OVX) and after sham surgery without castration or injection (n=6). Investigations included immunohistochemistry of estrogen and progesterone receptors and collagen fibres, Western blot analysis of collagen types I and III and counting of perianal vessels by light microscopy. The Animal Research Ethics Committee approved the study protocol.

Results

There was positive immunostaining for estrogen and progesterone receptors in the mucosa and for collagen types I and III in the submucosa in all samples. Western blot analysis showed that collagen type I increased after ovariectomy and then decreased in all groups. Collagen type I levels in sham treated animals were slightly higher than the experimental groups. The greatest decrease was observed in group 1. In contrast, collagen type III levels decreased after ovariectomy. Replacement of estrogen, progesterone and combination of estrogen and progesterone all increased the levels of type III collagen (Figure 1). Semi-quantitative measurement of type I/III collagen ratio, using signal intensity was 3:1 in ovariectomized animal and 1.6:1 in sham treated rats. Treatment with progesterone (1.3:1) produced a greater effect than either estrogen alone (1:1) or in combination with progesterone (1.2:1) (Figure 1). The differences were however, insignificant. The mean submucosal blood vessel count in the anal canal was significantly increased in sections of ovariectomized rats (14 \pm 2.6) compared with sham treated rats (6.2 \pm 2.5, p<0.006). The vessel count after estrogen replacement (9.3 \pm 1.7) was also significantly greater than that of sham (p= 0.04). The difference between the sham group and progesterone replacement (6 \pm 2.9) and a combination of estrogen and progesterone (4.5 \pm 1.3) was not significant.

Interpretation of results

Type I collagen levels increased significantly with ovariectomy but were normalized with treatment with estrogen and progesterone. Type III collagen levels decreased after ovariectomy. Administration of estrogen and progesterone appeared to restore level to near sham values. Type I/III collagen ratios increased markedly after ovariectomy. This appeared to be restored by both estrogen and progesterone administration either individually or in combination. Mean vessel count was significantly lower in sham compared to values in ovariectomized animals. However, while only estrogen treatment increased significantly the

number of vessels compared to sham animals, replacement with progesterone did not affect and in combination with estrogen reduced submucosal vessel number.

Concluding message

Estrogen and progesterone have synergistic effects on submucosal collagen types I and III and probably antagonistic effects on the perianal vascular plexus in the anal canal of adult female rats. The present results support for the first time the notion of an effect of both hormones on the submucosal connective and vascular tissues of the female anal canal. These experimental data may have a clinical impact on the management of climacteric women with idiopathic fecal incontinence.

References

1. Anorectal anatomy and physiology. *Gastroentrol Clin North Am* 2001, 30: 1-9.
2. Effects of ovariectomy and hormone replacement on collagen and blood vessels of the urethral submucosa of rats. *Urol Res* 2003; 31: 147-51.
3. Glutaraldehyde cross-linked collagen in the treatment of faecal incontinence. *Br J Surg* 1998; 85: 978-9.

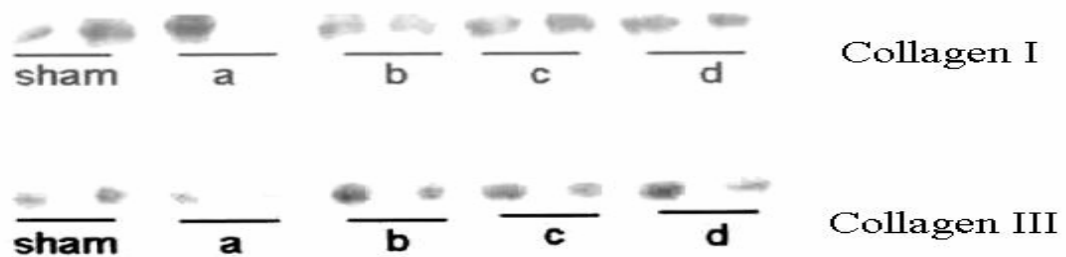


Figure 1

Western blot analysis of collagen types I and III in the anal submucosa of sham, controls [a] and groups 1 [b], 2 [c] and 3 [d]. Signal intensity for type I increased in controls [a] but decreased in all treatment groups [b,c,d]. Signal intensity for type III decreased in controls [a] but increased in all treatment groups [b,c,d].