Markers of collagen synthesis and degradation in urogenital tissue and serum from women with and without uterovaginal prolapse

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Diverging results have been published concerning collagen metabolism in uterovaginal prolapse (UP). We have investigated collagen turnover in urogenital tissue in urologically healthy women with (UP patients) and without UP or any history of UP (controls). Markers of collagen turnover, carboxy-terminal propeptide of type I procollagen (PICP), amino-terminal propeptide of procollagen III (PIIINP) and carboxy-terminal telopeptide of type I collagen (ICTP) were assayed in urogenital tissue homogenates and serum. Tissue and serum concentrations of collagen turnover markers were related to UP and to menopausal/estrogen status. UP patients were significantly older than the controls. UP patients had significantly higher tissue PICP and PIIINP and significantly lower tissue ICTP levels than the controls, but the difference in ICTP disappeared after matching for menopausal/estrogen status and age. There were no associations between tissue collagen turnover markers on the one hand and menopausal/estrogen status or age on the other. The higher tissue concentrations of PICP and especially PIIINP in tissue from women with UP compared to controls, suggest an increased collagen breakdown in UP. This pattern differs from that in stress urinary incontinent women without UP, where tissue levels of collagen turnover markers are low, indicating reduced collagen breakdown.

Keywords: uterovaginal prolapse; collagen turnover markers; urogenital tissue

Introduction

The content as well as the spatial organization of different types of collagen is the key to tissue strength and elasticity. Type I collagen is the predominant protein in mineralized bone but is also abundant in soft tissues, whereas Type III collagen only exists in soft tissue (Eriksen et al., 1993; Risteli and Risteli, 1993; Risteli et al., 1995). Several biochemical serum markers of the synthesis and degradation of collagens have been developed in order to find markers for bone metastasis or osteoporosis and/or for evaluation of estrogen treatment. In collagen synthesis, large parts from both the carboxy- and amino-terminal ends of the precursor molecule are split off and released into the extra cellular fluid. Assays of circulating carboxy-terminal propeptide of Type I procollagen (PICP) and of the carboxy-terminal telopeptide of Type I collagen (ICTP) are widely used for studying synthesis of Type I collagen and of the degradation of mature, trivalently crosslinked Type I collagen respectively (Hassager et al., 1992; Suvanto-Luukonen et al., 1997). The amino-terminal propeptide of procollagen III (PIIINP) is used for studying synthesis of new type III collagen.

Uterovaginal prolapse (UP) is a common pelvic support defect of low morbidity, but profoundly affecting the quality of life. In Sweden, ~6000 women will be operated every year for this disorder. Multiparity, especially with vaginal delivery, age and genetic predisposition are suggested risk factors for UP (Timonen et al., 1987; Ewies et al., 1997). The amino-terminal propeptide of procollagen III (PIIINP) is used for studying synthesis of new type III collagen. For Type I collagen, Liapis et al. (2001) reported similar levels in genital tissue from women with and without UP, whereas Ewies et al. (2003) report higher collagen Type III content in tissue from women with UP and this finding was supported by some similar studies (Gabriel et al., 2005; Moalli et al., 2005) but not by others (Lin et al. 2007). For total collagen, numerous workers have reported decreased tissue...
levels in UP (Stanosz et al., 1995; Jackson et al., 1996; Takano et al., 2002; Wong et al., 2003; Söderberg et al., 2004).

Concerning collagen turnover and structure, Jackson et al. (1996) also reported that there was no difference in the ratio between Types I and III collagen between women with and without UP but collagen from women with UP is considerably less acid-soluble, probably reflecting the maturity of the tissue. They also reported a marked increase in collagen metabolism due to the increased cathepsin and metalmatrix protease (MMP) levels, leading to loss of strength of the tissue. Similar findings are reported in the very recent paper of Phillips et al. (2006), who reported increased pro-MMP2 RNA expression in vaginal tissue from women with UP compared with tissue from healthy women. They further found very close correlations between the expressions of different MMPs in vaginal mucosa and uterosacral ligaments. Also, they discussed to what degree an increased collagen breakdown precedes the prolapse or represents a resistance to stretching within the uterosacral ligaments in UP.

In view of the partially diverging results referred to above and in order to get further insight about possible mechanisms behind UP, we have determined collagen turnover markers in urogenital tissue and in peripheral serum from urologically healthy women with UP and compared these data with those obtained from urologically healthy controls without UP.

Materials and Methods

Clinical material

Ethical approval was obtained for the study. A total of 48 women aged 46–87 years, referred for assessment at our department, were consecutively enrolled in the study. Women with malignant disease, diseases needing systemic glucocorticoid treatment, bishophosphate-treated osteoporosis and severe systemic diseases in general (cardiovascular, gastrointestinal, hepatobiliary, kidney, respiratory and neurological disease) were excluded from the study. Twenty-four of the women had UP as defined according to International Continence Society (ICS) standardization Stage II or more (Bump et al., 1996). They were shown to be continent by urodynamic investigation and controlled provocation with 300 ml saline in the bladder according to the ICS (Abrams et al., 2002). A further 24 women without UP, who proved urologically healthy in a simple cough provocation test with full bladder according to bladder scanning and had no history of stress urinary incontinence (SUI), served as controls. These women were a part of the clinical material used in our previous study on women with SUI and healthy controls (Edwall et al., 2005). Half of the controls were scheduled for hysterectomy, in most cases a vaginal operation, owing to uterine fibroids, bleeding disorders and in one case cervical stenosis and in another, pre-cancerous cervical epithelium. None of the patients with fibroids were on endocrine treatment regimens for their disease. The remainders of the controls were outpatients undergoing routine gynecological examination. Menopausal/estrogen status of the two groups is given in Table I.

Sample collection and analytical methods

Samples of suburethral tissue were taken from women with UP performing surgery for prolapse. The site of sampling was 10–20 mm beneath the external meatus of the urethra, just lateral to the midline. The samples were complete wall thickness—epithelium and connective tissue from sub/paraurethral location. Samples from the controls were taken as 6 mm punch biopsies at the same site. All tissue samples were taken by L.E. and were immediately placed on ice, frozen within less than 20 min and stored at −20 °C. Venous blood samples were taken before the day of surgery between 0900 and 1200 h. Serum was separated after centrifugation and stored at −80 °C until analysis.

Frozen tissue (100–500 mg) was cut into small slices on a block of dry ice and transferred to a pre-chilled (liquid nitrogen) capsule containing a Teflon-coated tungsten ball. The capsule was kept in liquid nitrogen for 2 min and thereafter shaken in a dismembranation apparatus (Retsch KG, Haan, Germany) at full speed for 2 min. The procedure was repeated once after intermediate freezing in liquid nitrogen. After thawing, the pulverized tissue was suspended in phosphate-buffered saline, pH 7.4, and the homogenate was kept frozen at −20 °C until analysis.

Serum and tissue concentrations of PICP, PIINP and ICTP were assessed by radioimmunoassay, using commercial kits obtained from Orion Diagnostica, Esbo, Finland, originally intended for assay of human serum. For assay of collagen turnover markers in urogenital tissue the frozen homogenates were thawed, mixed thoroughly and centrifuged at 2300 x g for 15 min in a refrigerated centrifuge. The supernatant was collected and used for assay of collagen turnover markers and total protein. Serial dilutions of tissue homogenate yielded dilution curves parallel to the standard curves. Detection limits and within- and between-assay coefficients of variation were 1.2 μg/l, 3 and 5% for PICP; 0.2 μg/l, 5 and 6% for PIINP and 0.5 μg/l, 5 and 6% for ICTP, respectively. Total protein was determined by the biuret method and tissue PICP was expressed as microgram of collagen turnover marker per milligram of total protein.

Statistical methods

Comparison between the groups was performed by Mann–Whitney U-test or t-test for unpaired observations according to distribution. Possible associations between tissue collagen turnover markers and menopausal/estrogen status (0, post-menopausal, no hormone replacement therapy (HRT); 1, post-menopausal, weak HRT (local estrogen or oral estriol); 2, post-menopausal, strong HRT (systemic estrogen substitution) and 3, premenopausal) were tested with Kruskal–Wallis test as well as with Spearman’s rank correlation test. The latter test was also used for other correlations. The significance level was set at P = 0.05. Normally distributed values are expressed as arithmetic mean ± SD; otherwise as median and range, except for parity which, for the sake of clarity, is expressed as arithmetic mean and range (geometric means cannot be used for data including zero).

Results

Data for menopausal/estrogen status, age, body mass index (BMI) and parity for the two categories of women are given in Table I. UP patients were significantly older than the controls. Urogenital tissue levels of collagen turnover markers are given in Fig. 1. T-PICP and T-PIINP levels in the UP patients were significantly higher and T-ICTP levels significantly lower than in the controls. Significant negative correlations to parity were found for T-PICP in the controls (r = −0.46, P < 0.05) and for T-ICTP in the UP patients (r = −0.42, P < 0.05); otherwise no associations between collagen turnover markers and parity were found. The effects of limiting the parity span are shown in Table II. When the upper parity limit was reduced to four, the significance of the difference in T-ICTP disappeared but remained for T-PICP and T-PIINP. Further reduction to three and two resulted in loss of the significance also for T-PICP but the difference in T-PIINP remained significant.

The differences in T-PICP and T-PIINP also remained significant when the two groups were matched for menopausal/estrogen status and age, with four untreated post-menopausal women, one post-

Table I. Menopausal/estrogen status, age, BMI and parity in patients with UP and in control patients.

<table>
<thead>
<tr>
<th></th>
<th>UP</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>N, total</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Post-MP, no HRT</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>Post-MP, weak HRT</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Post-MP, strong HRT</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Pre-MP</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Age (years)</td>
<td>67.0 ± 12.9 *** 53.3 ± 5.4</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.5 ± 3.5</td>
<td>24.8 ± 3.6</td>
</tr>
<tr>
<td>Parity</td>
<td>2.4 (0–7)</td>
<td>1.9 (0–5)</td>
</tr>
</tbody>
</table>

Weak HRT, local estrogen or oral estriol; strong HRT, systemic estrogen substitution; MP, menopausal; BMI, body mass index. Significance of difference between UP patients and controls denoted by ***P < 0.001.
A menopausal woman with weak HRT, three post-menopausal women with strong HRT and three premenopausal women in each group. T-PICP and T-PIIINP levels were still significantly higher in the UP patients: for T-ICTP 0.212 (0.022–0.804) versus 0.033 (0.010–0.204) mg/mg protein, \( P < 0.05 \) and for T-PIIINP 0.132 (0.045–0.615) versus 0.053 (0.025–0.118) mg/mg protein, \( P < 0.01 \), while the significance of the difference in T-ICTP disappeared. There were no significant differences in serum collagen markers (data not shown).

There were no significant associations, whatsoever, between age or menopausal/estrogen status and tissue collagen turnover markers, neither in the total clinical material nor in the two individual groups.

Discussion

Our findings have been made using a limited sample of clinical material and may perhaps be considered as preliminary. However, patient studies including strict selection criteria and qualified biochemical assays are usually performed on small clinical samples. With the exception of our study on SUI (Edwall et al., 2005), which included 71 patients and 31 controls, the median number (range) of patients in such studies cited in the present paper was 23(5–62) and of controls 15 (5–28) (Mäkinen et al., 1987; Jackson et al., 1996; Liapis et al., 2001; Barbiero et al., 2003; Ewies et al., 2003; Gabriel et al., 2005; Moalli et al., 2005; Phillips et al., 2006; Lin et al., 2007). Our patients and controls in the total clinical material differed with respect to age and to estrogen/menopausal status and it may therefore be argued that this constitutes a flaw in our study. However, as was found in our case–control study on SUI (Edwall et al., 2005) as well as in the present investigation, age or estrogen/menopausal status had no influence on the tissue concentrations of collagen turnover markers. Furthermore, the differences in T-PICP and T-PIIINP remained statistically significant after matching the two groups for estrogen/menopausal status. Concerning collagen Type III, Ewies et al. (2003) reported no influence of age and menopausal status on the expression of this collagen species in cardinal ligaments, whereas collagen Type I expression was directly related to age and menopausal status. However, it may be speculated that the levels of a collagen turnover marker may not always reflect the expression of the collagen type itself.

Multiparity, especially with vaginal delivery, is suggested as a risk factor for UP (Timonen et al., 1968; Porges and Smilen, 1994; Gill and Hurt, 1998). We found a significant negative correlation between T-ICTP and parity in the UP patients but we consider it as less likely that this association reflects some kind of mechanism by which multiparity constitutes a risk factor for UP. However, together with the finding of a significant negative correlation between T-PICP and parity in the controls and our findings of negative correlations between collagen turnover markers and parity in our previous study.

Table II. Concentrations of collagen turnover markers in urogenital tissue (T-PICP, T-PIIINP and T-ICTP) in urologically healthy women with uterovaginal prolapse (UP) and in urologically healthy women without UP (Controls) when the upper parity limit was restricted to 4, 3 or 2.

<table>
<thead>
<tr>
<th></th>
<th>UP</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–4-parous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>T-PICP, mg/mg protein</td>
<td>0.133 (0.030–0.804)*</td>
<td>0.105 (0.010–0.218)</td>
</tr>
<tr>
<td>T-PIIINP, mg/mg protein</td>
<td>0.132 (0.045–0.615)**</td>
<td>0.080 (0.017–0.188)</td>
</tr>
<tr>
<td>T-ICTP, mg/mg protein</td>
<td>0.014 (0.002–0.187)</td>
<td>0.027 (0.007–0.083)</td>
</tr>
<tr>
<td>0–3-parous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>21</td>
<td>23</td>
</tr>
<tr>
<td>T-PICP, mg/mg protein</td>
<td>0.127 (0.030–0.804)</td>
<td>0.105 (0.010–0.218)</td>
</tr>
<tr>
<td>T-PIIINP, mg/mg protein</td>
<td>0.134 (0.045–0.615)**</td>
<td>0.080 (0.017–0.188)</td>
</tr>
<tr>
<td>T-ICTP, mg/mg protein</td>
<td>0.015 (0.002–0.187)</td>
<td>0.027 (0.007–0.083)</td>
</tr>
<tr>
<td>0–2-parous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>T-PICP, mg/mg protein</td>
<td>0.127 (0.030–0.804)</td>
<td>0.115 (0.016–0.218)</td>
</tr>
<tr>
<td>T-PIIINP, mg/mg protein</td>
<td>0.141 (0.045–0.615)**</td>
<td>0.086 (0.017–0.188)</td>
</tr>
<tr>
<td>T-ICTP, mg/mg protein</td>
<td>0.019 (0.004–0.187)</td>
<td>0.029 (0.007–0.083)</td>
</tr>
</tbody>
</table>

The values shown are medians and (range). Significances of differences between UP patients and controls are denoted by *\( P < 0.05 \) and **\( P < 0.01 \).

Figure 1: Concentrations of collagen turnover markers in urogenital tissue (T-PICP, T-PIIINP and T-ICTP) in urologically healthy women with uterovaginal prolapse (UP) (\( N = 24 \)) and in urologically healthy women without UP (CTR) (\( N = 24 \)). Horizontal lines indicate (from bottom to top) the 10th, 25th, 50th (median), 75th and 90th percentiles. Significances of differences between UP patients and controls are denoted by *\( P < 0.05 \) and **\( P < 0.01 \).
on SUI (Edwall et al., 2005), it again suggests that pregnancy may affect urogenital tissue factors not only by purely mechanical means but also by changing collagen metabolism.

Although decreased T-ICTP levels suggest a reduced breakdown of collagen Type I, our finding of significantly elevated levels of T-PICP and T-PIIINP in the UP patients suggest an increased synthesis of collagen. An increased breakdown of collagen in UP has been suggested, based on the finding of increased MMP and cathepsin levels and increased MMP RNA expression in urogenital tissue from women with UP (Jackson et al., 1996; Phillips et al., 2006). The elevated levels of T-PICP and T-PIIINP in our UP patients may therefore reflect an increased collagen synthesis following breakdown in UP. Our results suggest that an increased collagen synthesis in UP may primarily be valid for collagen Type III. The difference in T-PIIINP was more substantial than for the two other markers and remained significant also after limiting of parity span and in the small matched groups. The markers of collagen Type I turnover are more difficult to interpret and the differences more or less disappeared after limiting of parity span and after matching. The enzymes responsible for collagen breakdown in urogenital tissue prefer newly formed collagen, thus inhibiting remodeling (Jackson et al., 1996). An increased collagen breakdown, leading to lower total collagen levels, would also be in accordance with the finding of low tissue total collagen in UP in other studies (Stanisz et al., 1995; Jackson et al., 1996; Takano et al., 2002; Wong et al., 2003; Söderberg et al., 2004) and may affect strength and elasticity of genital tissue in UP.

In contrast to the data on total collagen referred to above, Ewies et al. (2003) reported higher collagen Type III content in tissue from women with UP and this finding was supported by some similar studies (Gabriel et al., 2005; Moalli et al., 2005). Women with concomitant SUI were included in the clinical material used by Moalli et al. (2005) and data on concomitant SUI are lacking in the two other studies (Ewies et al., 2003; Gabriel et al., 2005). On the other hand, concomitant SUI was an exclusion criterion in the study of Jackson et al. (1996) as well as in the present investigation. There are indications for a reduced turnover of collagen Type III in SUI, which may cause an accumulation of this collagen species (Edwall et al., 2005 and references cited therein).

By using ‘clean’ well-defined groups of patients and controls, our studies suggest quite different patterns of collagen turnover in UP and SUI, with increased collagen synthesis in the former and decreased breakdown in the latter condition (Edwall et al., 2005). In our study on SUI patients from 2005, we found decreased concentrations of collagen turnover markers in urogenital tissue from SUI patients, indicating a decreased collagen breakdown. Our finding of higher tissue concentrations of PICP and PIIINP in genital tissue from women with UP than in controls suggests an increased collagen synthesis in UP. This may negatively affect the strength and elasticity of urogenital tissue; however to what degree an increased collagen breakdown is a cause or a result of the UP remains to be demonstrated.

Acknowledgements

The text in the Introduction dealing with the background for the use of collagen turnover markers and the Methods text in the Materials and Methods section have previously been published in two papers from our group (Edwall et al., 2005, 2007) and are included in the present paper with permission from the publisher of these papers, Wiley-Liss, Inc.

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