Increased concentrations of cathepsin D in peritoneal fluid from women with endometriosis

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To assess the release of the proteolytic enzyme cathepsin D in endometriosis, concentrations in peritoneal fluid and serum were measured by ELISA in 54 women with (n = 33) and without (n = 21) endometriosis. Surgery was scheduled in either the proliferative or secretory phase of the menstrual cycle. The concentrations of cathepsin D in the peritoneal fluid were markedly elevated in the endometriosis patients (median 58 ng/ml, interquartile range 0–166 ng/ml) as compared to the controls (5 ng/ml, 0–86 ng/ml), especially in women with late stage disease (n = 19, stages III/IV) and in those not undergoing gonadotrophin-releasing hormone (GnRH) agonist therapy (n = 15).

No significant difference was determined in cathepsin D concentrations of the serum from women with and without endometriosis. We conclude that cathepsin D is an important factor that may contribute to the pathogenesis of endometriosis, possibly by promoting digestion of extracellular matrix proteins. These results have implications for the therapeutic efficacy of GnRH agonists.

Key words: cathepsin D/endometriosis/gonadotrophin-releasing hormone/peritoneal fluid

Introduction

Cathepsin D is an aspartyl acid protease widely distributed in animal and human cells. It is initially synthesized as an inactive 52 kDa precursor (pro-cathepsin D) and proteolytic removal of the aminoterminal pro fragment then results in an enzymatically active 48 kDa heterodimer consisting of two chains of 14 and 34 kDa (Capony et al., 1987; Fortenberry and Chirgwin, 1995). Cathepsin D is induced by oestrogens in mammary cancer cells where its concentration is correlated with a high risk of metastasis (Sypartos et al., 1989). Its gene expression is also differentially regulated by sex-steroid hormones in human uterine (Moulton and Boening, 1983; Maudelonde et al., 1990) and breast cancer cells (Cavailles et al., 1989; Brouillet et al., 1991).

Endometriosis features progressive growth and invasion which is oestrogen-dependent (Rock, 1992). Several lines of evidence suggest that cytokines, chemokines, and growth factors are involved in the maintenance and development of endometriotic lesions. Concentrations of several molecules are known to be elevated in the peritoneal fluid of endometriosis patients, examples being interleukins (IL) and vascular endothelial growth factor (VEGF) (Fakih et al., 1987; McLaren et al., 1996). Recently, various elevated factors in the peritoneal fluid, for instance insulin-like growth factors (IGF), IGF-binding proteins and leptin have also been reported (Kim et al., 2000; Matarese et al., 2000). Since cathepsin D is induced by growth factors in breast cancer cells (Cavailles et al., 1989) and in the uterus (Maudelonde et al., 1990), we speculated that its concentrations in peritoneal fluid might be similarly elevated in women with endometriosis.

A previous study showed that in endometriosis patients, endometriotic tissue levels of cathepsin D are significantly higher than those in eutopic endometrium, suggesting potential importance for implantation and invasive growth (Bergqvist et al., 1996). However, to our knowledge, no study of cathepsin D levels in peritoneal fluid and serum of endometriosis patients has been published previously. Moreover, the function of cathepsin D under the influence of gonadotrophin-releasing hormone (GnRH) analogues has yet to be elucidated, despite the hypo-oestrogenism associated with the GnRH treatment, and the suggestion (Maudelonde et al., 1990) that cathepsin D is induced by progesterone in the human endometrium. Therefore, the present study was designed to assess the menstrual cycle and endometriosis stage variations in proteolytic enzyme cathepsin D levels in peritoneal fluid and serum from women with this disease.

Materials and methods

Fifty-four women aged 21–48 years with endometriosis (n = 33) and without endometriosis (n = 21) were enrolled in this study. The study
The peritoneal fluid of the women with endometriosis (n = 33) contained significantly higher (P < 0.001) concentrations of cathepsin D (median 58 ng/ml, interquartile range 0–166 ng/ml) than did peritoneal fluid from the controls (n = 21), i.e. women without endometriosis (5 ng/ml, 0–86 ng/ml) by the Mann-Whitney test.

The endometriosis patients were staged from I to IV depending on the severity of disease based on the Re-AFS classification. The stage distribution during surgery was as follows: total numbers (GnRH agonist-treated numbers): stage I, 8 (3); stage II, 6 (3); stage III, 11 (5); stage IV, 8 (4). There was significant variation (P < 0.001) in cathepsin D levels between the control group (n = 21), the endometriosis stages I/II group (n = 14) and the endometriosis stages III/IV group (n = 19) by the Kruskal-Wallis test (Table I and Figure 1A).

In these patients, elevated cathepsin D levels in the peritoneal fluid were noted in the stages III/IV group (n = 19) as compared to the stages I/II group (n = 14) (Figure A, P < 0.05).

No significant changes in cathepsin D during the menstrual cycle were observed in any groups of the controls or endometriosis patients (Table I and Figure 1B). With respect to the pre-operative GnRH agonist treatment, peritoneal fluid concentrations of cathepsin D were lower for the GnRH agonist-treated patients (n = 15) than for the untreated cases (n = 18) (Figure 1B, P < 0.01).

Cathepsin D levels in the serum of the endometriosis patients (n = 27; median 0 ng/ml, interquartile range 0–2 ng/ml) were not significantly different from those of the control group (n = 20; 0 ng/ml, 0–2 ng/ml) (Table I).

### Discussion

In the present study, we demonstrated that the concentration of cathepsin D in peritoneal fluid is significantly elevated in untreated women with endometriosis compared to the GnRH agonist-treated patients or the controls.

Recent findings suggest that concentrations of several molecules are elevated in the peritoneal fluid of endometriosis patients. We have shown that secretory leukocyte protease inhibitor is released from endometriotic tissue itself (Suzumori et al., 1999) and it is reported that this is also the case for IL-6 (Tseng et al., 1996). Previously it has been found that cathepsin D levels (median 460 pmol/mg DNA) in endometriotic tissues are significantly higher than in eutopic endometrium (Bergqvist et al., 1996). Our present findings suggest that cathepsin D production from spreading endometriotic lesions may be high so that an elevated level in the peritoneal fluid becomes recognized in late stages of endometriosis, e.g. stages III and IV based on the Re-AFS classification. Thus, the proteolytic enzyme cathepsin D could be involved in the spreading and development of endometriosis and its

### Table I. Characteristics of patients and concentrations of cathepsin D in peritoneal fluid and serum

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Agea (years)</th>
<th>Cathepsin D concentrationb (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peritoneal fluid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>21</td>
<td>37 ± 1.8</td>
</tr>
<tr>
<td>Endometriosis</td>
<td></td>
<td>5 (0–86)c</td>
</tr>
<tr>
<td>Stages I and II</td>
<td>14</td>
<td>35 ± 1.3</td>
</tr>
<tr>
<td>Stages III and IV</td>
<td>19</td>
<td>36 ± 2.0</td>
</tr>
<tr>
<td>Proliferative phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>36 ± 1.9</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>19</td>
<td>4 (0–125)</td>
</tr>
<tr>
<td>Secretory phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>11</td>
<td>38 ± 2.9</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>14</td>
<td>5 (0–86)</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>20</td>
<td>35 ± 1.6</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>27</td>
<td>0 (0–2)</td>
</tr>
</tbody>
</table>

aValues are means ± SEM.
bValues are medians (interquartile range).

*P < 0.001, **P < 0.05 (determined by the Mann-Whitney test).
menstrual cycle was noted (P: the proliferative phase; S: the secretory phase) in any groups marked **. No significant difference was observed between the controls and the GnRH agonist-treated patients. However, the cathepsin D levels of the untreated patients (n = 18; n = 10, the proliferative phase; n = 8, the secretory phase) were significantly higher than those from patients receiving GnRH agonist treatment (n = 15; n = 9, the proliferative phase; n = 6, the secretory phase, P < 0.01).

measurement in the peritoneal fluid could be a good marker for the severity of the disease.

There is currently considerable interest in optimal timing of surgery according to the phase of the menstrual cycle in terms of tumour biology (Badwe et al., 1995; Holdaway et al., 1997) and prognosis (Veronesi et al., 1994; Mohr et al., 1996). Since endometriotic lesions are influenced by cyclic changes in ovarian steroids, we examined whether the cathepsin D concentration varies with the phase of the menstrual cycle. Although cathepsin D is induced by oestrogens in mammary cancer cells, the lack of correlation between peritoneal fluid cathepsin D levels and the menstrual phase, regardless of the presence of endometriosis, indicates that hormonal induction of cathepsin D may not be a major factor. Several lines of evidence suggest that tissue cathepsin D concentrations appear to be correlated more with tumour invasiveness than with hormonal responsiveness, independent of regulation by oestrogen (Spyratos et al., 1989). Therefore, we suggest that invasion with endometriosis may occur locally with some contribution of cathepsin D regardless of ovarian steroids. However, since peritoneal fluid concentrations of cathepsin D in the GnRH agonist-treated patients were relatively low, it is likely that induction of hypo-oestrogenism may therefore reduce the local invasiveness.

In clinical oncology, serum and tissue levels of cathepsin D have been widely studied with regard to a potential prognostic value, particularly in breast cancer patients (Westley and May, 1996; Pujol et al., 1999). Overexpression of cathepsin D in tumour tissue was thereby observed to be an independent marker for identifying patients at risk of disease progression. However, elevated cathepsin D serum values have only been recognized in pancreatitis or liver cirrhosis patients, and not in individuals with breast cancer (Brouillet et al., 1991; Leto et al., 1997). Recent reports have in fact suggested that serum cathepsin D concentrations are not elevated in most cancer patients, regardless of the clinical stage or the tumour size (Leto et al., 1997; Westhoff et al., 1998). In spite of high cathepsin D levels in endometriotic tissue (Bergqvist et al., 1996) and our present findings for peritoneal fluid, our data also indicate that there is no potential for diagnostic use of serum cathepsin D levels for patients with endometriosis.

In malignant cells, cathepsin D has been found to be overexpressed or abnormally processed, resulting in its cytoplasmic accumulation and excessive secretion (Strojan et al., 1998). Several findings suggest that it may be related to local invasion and metastasis of tumour cells, and it is able to initiate proteolytic events resulting in degradation of basement membrane and extracellular matrix components (Scambia et al., 1991; Strojan et al., 1998). In endometrial adenocarcinomas, the levels of cathepsin D are higher than in normal endometrium and correlate positively with loss of differentiation and the degree of myometrial invasion (Nazeer et al., 1992). Our data point to elevated cathepsin D levels in the peritoneum due to proteolytic invasion of endometriotic lesions.

We conclude that cathepsin D is an important factor that may contribute to the pathogenesis of endometriosis, possibly by digesting extracellular matrix proteins. This information has implications for the therapeutic efficacy of GnRH agonists.

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N. Suzumori et al.


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