Activities of Dipeptidyl Peptidase II and Dipeptidyl Peptidase IV in Synovial Fluid from Patients with Rheumatoid Arthritis and Osteoarthritis

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We examined the activities of peptidases in synovial fluid from patients with rheumatoid arthritis (RA) and osteoarthritis (OA). Dipeptidyl peptidase IV (DPP IV) activity was lower in synovial fluid from patients with RA, in contrast to the increase of DPP II activity in synovial fluid, as compared with OA. The DPP II/DPP IV ratio for synovial fluid was significantly higher in patients with RA than in patients with OA. A significant correlation was observed between the DPP II/DPP IV ratio for synovial fluid from patients with RA and the amount of C-reactive protein reaction. These results may be useful in the diagnosis of joint effusion of unknown origin.

Dipeptidyl peptidase II (EC 3.4.14.2; DPP II) and dipeptidyl peptidase IV (EC 3.4.14.5; DPP IV), both serine peptidases, cleave a dipeptide from unsubstituted NH2 termini of dipeptide derivatives.5 DPP II was first identified in bovine anterior pituitary extracts by McDonald and co-workers (1, 2). DPP II preferentially hydrolyzes Lys-Ala-2-naphthylamide at pH 5.5, but also cleaves the N-terminal dipeptide from dipeptide 2-naphthylamides and tripeptides with a penultimate alanine or prolyl residue (1, 2). DPP IV was discovered in rat liver and kidney by Hopen-Havu and Glenner (3) in 1966. DPP IV hydrolyzes peptides with an N-terminal sequence of X-pro-Y- to yield X-pro and Y- at pH 8.0 (3). DPP II differs from DPP IV in its subcellular localization and pH optimum.

The origin of the DPP II and DPP IV is not yet clear, but the activities of the two enzymes may reflect changes of the dipeptides in various tissues. As reported previously (4, 5), DPP IV activities, with Gly-Pro-4-methylcoumarinamide (Gly-Pro-MCA) used as substrate, were normal in serum from patients with cancer, rheumatoid arthritis (RA), and systemic lupus erythematosus. In contrast, DPP II activities in serum from patients with cancer, RA, and systemic lupus erythematosus were found to be higher than that in normal serum when Lys-Ala-MCA was used as the substrate. Thus, the DPP II/DPP IV ratio tended to increase in serum from patients with cancer, RA, and systemic lupus erythematosus.

Post-proline cleaving enzyme (PPCE, prolyl endopeptidase, EC 3.4.21.26), also a member of the same family, cleaves -X-Pro-Y- to -X-Pro and Y-. PPCE is capable of degrading many neuropeptides (6). Succinyl-Gly-Pro-MCA is one of the most sensitive substrates for PPCE (7).

Various enzymes had been found increased in synovial fluid from patients with RA and correlated with the clinical parameters (8–11).

In this study, we have measured DPP II, DPP IV, and PPCE activities in synovial fluids from patients with RA and OA. In RA, we also compared the DPP II/DPP IV ratio with the duration of the disease up to the time of the enzyme assay and the extent of the disease as reflected in erythrocyte sedimentation rate (ESR) and the C-reactive protein (CRP) reaction.

Materials and Methods

**Samples.** Synovial fluids were aspirated from knee joints for diagnostic or therapeutic purposes. Thirty specimens of synovial fluid were obtained from 30 patients with classical or definite RA, diagnosed according to the diagnostic criteria of the American Rheumatism Association (12).

Thirty specimens of synovial fluid were also aspirated from 30 patients with OA, having painful joints with radiological evidence of joint-space narrowing and subchondral bone reaction. All these patients had normal hepatic function.

The male/female ratio was 4/26 for RA, 3/27 for OA. The mean age of the patients was 58.7 (range 31–84) years for the RA group, and 64.3 (41–95) years for the OA group.

The synovial fluids were collected and immediately stored at −80°C. Before assay, the synovial fluids were centrifuged at 700 × g for 15 min, and the enzymatic activities were measured in the supernates.

**Substrates for enzyme assay.** The following substrates were obtained from the Peptide Institute, Protein Research Foundation, Minoh, Osaka 562, Japan: 7-amino-4-methylcoumarin (AMC), Lys-Ala-MCA, Gly-Pro-MCA, and succinyl-Gly-Pro-MCA.

**Buffers.** We used the following buffers: "universal" buffer (0.2 mol of sodium borate and 0.05 mol of citrate per liter, adjusted to pH 5.3 with 0.1 mol/L sodium phosphate buffer) for the DPP II assay, glycine-NaOH buffer (0.15 mol/L each, pH 8.7) for DPP IV, and sodium phosphate buffer (0.2 mol/L, pH 6.8) containing 1 mmol of EDTA per liter for PPCE.

**Enzyme activity.** All enzyme activities were assayed fluorometrically by measuring the enzymatic formation of AMC. DPP II activity was measured fluorometrically with Lys-Ala-MCA as substrate. The reaction mixture (total volume 0.1 mL) contained 40 μmol of universal buffer, 25 μmol of 8 mmol/L Lys-Ala-MCA, 10 μmol of 10 mmol/L o-phenanthroline, and 25 μL of sample. Instead of sample, the blank and standard tubes respectively contained 25 μL of water or 15 μL of water plus 10 μL (1.00 nmol) of 100 μmol/L AMC reagent. The control tube contained no sample. All tubes were incubated at 37 °C for 30 min. The reaction was then stopped by adding 1.0 mL of acetate buffer (1 mol/L, pH 4.2), and 25 μL of sample was added to the control tube. The fluorescence intensities of the sample (Sa), control (C), standard (St), and blank (B) were measured at 460 nm in a Shimadzu RF-500 spectrofluorometer (excitation...
Table 1. Peptidase Activities (Mean ± SE) in Synovial Fluids from Patients with RA and OA

<table>
<thead>
<tr>
<th>Group</th>
<th>No. samples</th>
<th>DPP II (μU per mg protein)</th>
<th>DPP IV (μU per mg protein)</th>
<th>PPCE (μU per mg protein)</th>
<th>(DPP II/DPP IV) × 100</th>
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<tbody>
<tr>
<td>RA</td>
<td>30</td>
<td>47.0 ± 3.6 (14.4-84.2)</td>
<td>548.6 ± 28.3 (348.3-958.0)</td>
<td>66.6 ± 7.4 (24.2-159.7)</td>
<td>8.82 ± 0.74 (2.67-17.91)</td>
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<tr>
<td>OA</td>
<td>30</td>
<td>32.3 ± 2.3* (11.3-66.4)</td>
<td>702.6 ± 41.2* (373.0-194.1)</td>
<td>60.0 ± 4.0 (18.9-101.0)</td>
<td>4.77 ± 0.39** (2.54-12.39)</td>
</tr>
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</table>

Significant differences indicated between RA and OA at *P < 0.01, **P < 0.001.

wavelength 380 nm). AMC liberated by the enzyme reaction was calculated as follows:

\[
\text{SA} - C \times \frac{1\, \text{nmol}}{30\, \text{min}} \times \frac{1}{0.000025}\]

\[
= \frac{4(SA - C)}{3(St - B)} \text{U/L in sample (37 °C)}
\]

DPP IV and PPCE activities were also measured fluorometrically with Gly-Pro-MCA and succinyl-Gly-Pro-MCA as respective substrates, as described previously (13).

Discussed by use of the biuret reaction (14). ESR and CRP were determined as described previously (15, 16).

Results

Table 1 shows the activities of DPP II, DPP IV, and PPCE (μU per mg protein) and the DPP II/DPP IV ratio for synovial fluids from patients with RA and OA.

DPP II activity in patients with RA was significantly (P < 0.01) higher than in patients with OA, whereas DPP IV activity was significantly (P < 0.01) lower in patients with RA than OA. Thus, the DPP II/DPP IV ratio was significantly increased (P < 0.001) in patients with RA compared with those in patients with OA. No significant difference in PPCE activity was observed between patients with RA and OA.

The DPP II/DPP IV ratio for synovial fluids from patients with RA showed no significant correlation with either the duration of the disease or the ESR. However, there was a significant correlation between the DPP II/DPP IV ratio for synovial fluids (y) from patients with RA and the amount of CRP reaction (x): y = 0.21x ± 0.43 (r = 0.51, P < 0.01).

Discussion

In our previous reports, the activities of DPP II and DPP IV in serum and the DPP II/DPP IV ratio were found to be diagnostic indices in some diseases. DPP II activity, with Lys-Ala-2-naphthylamide used as substrate, was abnormally increased in serum from patients with thromboembolic diseases, myocardial infarction, diabetes mellitus, and alcoholism (17). Serum DPP IV activity with X-Pro-p-nitroanilides used as substrates was abnormally increased in patients with hepatobiliary diseases and decreased in patients with various solid-tissue and blood cancers (4, 18, 19) or collagen diseases such as RA and systemic lupus erythematosus. A significant inverse correlation was observed between the DPP IV activity in serum from patients with RA and the duration of RA (20).

We found DPP II activity in synovial fluid from patients with RA to significantly exceed that of patients with OA. In contrast, DPP IV activity was significantly lower in synovial fluid from patients with RA than in that from those with OA. Thus, the DPP II/DPP IV ratio was significantly greater for synovial fluid from patients with RA than for those with OA. These results could be interpreted as reflecting a difference between RA and OA in the process of collagen breakdown in the joint. In fact, breakdown of collagen in RA patients is caused by inflammation; in contrast, in OA patients collagen breakdown occurs in the cartilage on the surface of the joint and is not inflammatory in nature.

Moreover, in the present study, significant correlations were observed in RA patients between the DPP II/DPP IV ratio for synovial fluid and the CRP reactivity in serum. The relation between the serum CRP reactivity and the DPP II/DPP IV ratio for synovial fluid from patients with RA indicates that the ratio may be a biochemical index to the severity of RA.

As regards the role of these enzymatic changes, it is not clear whether the observed changes are the result of or cause of the disease. The origin of these enzymes is not clear (8). West et al. (8) suggested that increased enzyme activity in the joint fluid might result from several mechanisms. The mechanism of increase in the DPP II/DPP IV ratio in synovial fluid from patients with RA remains to be clarified. Nevertheless, these results may be empirically useful in the diagnosis of joint effusion of unknown origin and in the evaluation of treatment for RA.

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References