Urinary excretion of glucosyl-galactosyl pyridinoline: a specific biochemical marker of synovium degradation

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Abstract

Objective. Glucosyl-galactosyl pyridinoline (Glc-Gal-PYD), which has been identified in urine, is a glycosylated analogue of pyridinoline. The tissue distribution of this molecule has not been yet determined and its utility as a potential biochemical marker of joint degradation in patients with joint diseases has not been investigated.

Methods and results. In this study, we demonstrate that Glc-Gal-PYD is abundant in human synovium tissue, absent from bone and present in minute amounts in cartilage and other soft tissues, such as muscle and liver. Using an ex vivo model of human joint tissue degradation, we found that Glc-Gal-PYD is released from synovium tissue, but not from bone and cartilage. The urinary level of Glc-Gal-PYD was increased by 109% in patients with rheumatoid arthritis (RA) compared with healthy adults, but was normal in patients with Paget’s disease of bone. In addition, Glc-Gal-PYD was higher in those patients with destructive disease, as assessed by X-rays of the joints, than in those with non-destructive RA.

Conclusion. Glc-Gal-PYD may be useful for the clinical investigation of patients with joint disease.

Key words: Synovium, Cartilage, Bone, Rheumatoid arthritis, Osteoarthritis, Pyridinoline, Glycosylation, Collagen.

The synovial membrane plays a major role in rheumatoid arthritis (RA) by producing inflammatory cytokines such as interleukin 1 and tumour necrosis factor α, which are involved in the breakdown of cartilage matrix and in the deterioration of subchondral bone [1]. At the present time, diagnosis and monitoring of inflammatory joint diseases is performed by scoring the pain and the mobility impairment caused by the joint destruction. Inflammatory markers such as C-reactive protein (CRP), rheumatoid factor and erythrocyte sedimentation rate (ESR) are frequently used for clinical diagnosis, but they have the disadvantages of not being specific for joint diseases and being poorly correlated to joint destruction [2–3]. The availability of specific markers reflecting the turnover of cartilage, synovium and bone is likely to be useful for a better understanding of joint tissue pathophysiology. Urinary excretion of pyridinium cross-links, especially deoxypyridinoline (DPD) and associated type I collagen telopeptides, is commonly used to monitor bone resorption [4]. Several markers reflecting cartilage and synovium tissue turnover have been reported, including fragments of aggregan and type II collagen, cartilage oligomeric matrix protein and glycoprotein YKL-40 [5–11], but their clinical utility remains uncertain [12]. Serum hyaluronan may be a useful marker of synovium metabolism, but it is not specific for synovium tissue, being also present in articular cartilage, the vitreous body of the eye, and the skin. To our knowledge, there is no specific marker reflecting the destruction of the synovium tissue. Pyridinoline (PYD) and DPD are trivalent structures which form the mature cross-links of type I, type II and type III fibrillar collagen. In contrast to DPD, which is present mainly in bone [13–14], PYD is particularly abundant in bone, cartilage and synovium [15–16]. The urinary excretion of pyridinium cross-links has been investigated as a potential marker of joint degradation in RA [17–19] and osteoarthritis [18–21]. Several studies have shown an increase in the excretion of total PYD and, to a lesser extent, of total DPD in patients with RA. PYD has also been shown to correlate with CRP levels, radiological severity and ESR. Recently, it was shown that serum levels of PYD and DPD were significantly higher in patients with RA than in healthy controls and were significantly correlated with disease activity [22]. Although the concentration of PYD is higher in patients with RA than in controls, it is submitted 6 March 2000; revised version accepted 2 September 2000. Correspondence to: E. Gineyts, INSERM U403, Hôpital E. Herriot, pavillon F, place d’Arsonval, 69437 Lyon cedex 03, France.
of limited value for the investigation of joint diseases because of its lack of specificity for a specific tissue of the joint.

A glycosylated analogue of PYD, glucosyl-galactosyl PYD (Glc-Gal-PYD) has been identified in urine [23–24]. It is the maturation product of two hydroxylsine residues from the C- or N-telopeptides of collagen with a glycosylated hydroxylsine from the z-helix of another collagen molecule. Little information is available on Glc-Gal-PYD concentrations. In women with postmenopausal osteoporosis, no significant increase in urinary excretion of Glc-Gal-PYD has been reported, contrasting with a 16% and 24% increase in free PYD and DPD respectively [23]. Although the presence of galactosyl PYD (Gal-PYD) in cartilage and bone [25] has been reported, no disaccharide derivatives of PYD have been isolated from connective tissues. Furthermore, the content of these compounds in synovium has not yet been investigated.

In this study, we quantified the content of Glc-Gal-PYD in several human tissues, including cartilage, bone and synovium, and investigated its urinary levels in healthy adults and patients with bone and joint disease.

Subjects and methods

Patients

Twenty healthy premenopausal women aged 31–39 yr with regular vaginal bleeding and 35 postmenopausal women aged 50–78 yr were recruited. None of the women was taking any medication known to influence calcium metabolism, including contraceptive pills in premenopausal women and hormone replacement therapy in postmenopausal women. All women were randomly selected from the OFELY (prospective study of the determinants of bone loss in women) study cohort, which comprises 1039 female volunteers 30–89 yr of age, taken from the regional section of a health insurance company (MGEN). Forty-one healthy men drawn from a large prospective study of the determinants of bone loss (MINOS) were also studied. None of the control subjects had any sign of joint disease. Thirteen patients (five women and eight men, mean age 69 yr) with active Paget’s disease but free of joint involvement were also studied. For nine of them, urine was also obtained 10 days after a single i.v. injection of the bisphosphonate zoledronate (400 mg). Twenty-seven patients (20 women and seven men, mean age 56 yr) with RA (disease duration 10.5 ± 6.9 yr) were also studied. They were divided into two groups according to the severity of joint destruction, as assessed by X-rays. Patients with destructive RA (n = 12) had long-standing severe RA, characterized by joint space narrowing and extensive bone erosions with multijoint involvement. The patients with non-destructive RA (n = 15) also had long-standing disease but were without joint damage or had radiographic evidence of mild joint damage. The patients were classified as having destructive or non-destructive RA by a trained rheumatologist who was blind to the patients’ values for biochemical markers.

Preparation of Glc-Gal-PYD standard

Glc-Gal-PYD was isolated from a pooled urine sample collected from children aged 2–10 yr. Pooled urine was passed through filters with a cut-off of 1.2 µm (Serum Capsule, Pall Gelman Laboratory, Ann Arbor, MI, USA) and 0.2 µm (SuporCap®, Pall Gelman Laboratory) and then freeze-dried. The urine powder was diluted to obtain 10-fold concentrated urine and mixed with acetic acid, 1-butanol and CF1 (Whatman, Maidstone, UK) cellulose powder. The urine:acetic acid:1-butanol ratio was 1:1:4. The slurry was passed through a filter, and the cellulose recovered was suspended in a 4:1:1 solvent mixture (1-butanol:acetic acid:water). After repeating this washing step 10 times, the cellulose was suspended in water to release pyridinium cross-links. The solution was concentrated and then chromatographed on a column (90 x 1.5 cm) of Sephadex G-10 (Pharmacia Biotech, Upplands, Sweden) and eluted in 10% (vol/vol) acetic acid, and fractions of 5 ml were collected. The 12 fractions of highest molecular weight, containing Glc-Gal-PYD, were pooled, freeze-dried and dissolved in 20% (vol/vol) n-heptafluorobutyric acid (HFBA) solution. Glc-Gal-PYD was separated by reverse-phase high-performance liquid chromatography (HPLC) on an UltraspHERE C18, 5 µm beads diameter, 250 x 10 mm column (Beckman Coulter Inc, Fullerton, CA, USA), protected by a Browlec¹⁷ RM-18 guard column, 7 µm beads diameter 15 x 3 mm (Applied Biosystems Inc, Foster City, CA, USA). The separation was performed isocratically at a flow rate of 3 ml/min in 17% acetonitrile and 0.15% HFBA solution. The effluent was monitored for fluorescence with emission at 395 nm and excitation at 297 nm, using a highly sensitive fluorescence detector (FP-920, Jasco Inc, Tokyo, Japan).

Electrospray mass spectrometry

For mass-spectral analysis, the isolated Glc-Gal-PYD was further purified by chromatography on a cellulose phosphate column (0.5 x 2 cm) that had been equilibrated with 1 m citrate buffer, pH 3.5. The column was washed with 1 ml citrate buffer, pH 3.5, and Glc-Gal-PYD was eluted with 0.5 M hydrochloric acid. Glc-Gal-PYD was structurally identified by its molecular mass deduced from negative ion mode electrospray ionization–mass spectrometry using a Platform spectrometer (Micromass, Manchester, UK). The Glc-Gal-PYD sample was dissolved in 50% methanol (vol/vol) and loaded at 10 µl/min, and spectra were recorded with a cone voltage at −70 V.

Tissue pyridinium content

Samples of human tissues, including bone, cartilage, synovium, skeletal muscle and liver, were obtained from apparently normal subjects during post-mortem autopsy. They were cleaned, cut into small pieces with a scalpel
and finely ground in a Spex Freezer-Mill (Spex Certi Prep, Metuchen, NJ, USA). Aliquots of powdered tissue (20 mg wet weight/ml) were hydrolysed with 6 M hydrochloric acid at 110°C for 20 h or with 2 M NaOH at 110°C for 5, 10, 15 or 20 h. Pyridinium cross-links were extracted from hydrolysates by cellulose CF1 partition column chromatography. Separation of the different forms of pyridinium cross-links was performed by HPLC. Samples were dissolved in 1% (vol/vol) HBFA and analysed by reverse-phase HPLC on an Ultrasphere C18, 5 μm beads diameter, 250 × 10 mm column (Beckman Coulter Inc, Fullerton, CA, USA), protected by a Browlee™ RP-18 guard column, 7 μm beads diameter, 15 × 3 mm (Applied Biosystems, Inc, Foster City, CA, USA) at a flow rate of 1 ml/min, with isocratic elution with 5.5 or 10% acetonitrile in 0.15% HBFA for alkaline and acid hydrolysates respectively. The effluent was monitored for fluorescence with emission at 395 nm and excitation at 297 nm.

Determination of pyridinium cross-links in culture medium of human tissue

Samples of human tissue, including bone, synovium, and cartilage, were obtained at the time of surgery for total hip replacement from patients with osteoarthritis. Culture of synovium, bone and cartilage explants was performed as described previously [26]. Briefly, tissue samples were cut into pieces of 2–5 mm³ and cultured in complete RPMI 1640 medium (Gibco, Grand Island, NY, USA) buffered with 20 mM HEPES [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] (Gibco) and supplemented with 5% calf serum (Techgen, Les Ulis, France), 2 mM l-glutamine (Gibco), 100 U/ml of penicillin and 50 μg/ml of gentamicin (Techgen). Human recombinant interleukin 1β (1 ng/ml) (Sigma, St Louis, MO, USA) was added on day 1 to synovium, bone and cartilage cultures. Plasminogen (5 mm) (Sigma) was added to cartilage culture only. Culture volume was 6 ml per well in six-well tissue culture plates (Falcon, Oxnard, CA, USA). After 5 days of culture at 37°C in a humidified environment of 5% carbon dioxide and 95% air, culture medium was collected and frozen for assessment of collagen degradation products.

Tissue culture media were collected and 5 ml of filtrate was subjected to cellulose CF1 partition column chromatography. Eluted material was analysed for free pyridinium cross-links by HPLC as described above for alkaline hydrolysates.

Urinary assay of total PYD, total DPD and Glc-Gal-PYD

The total excretion of pyridinium cross-links (total PYD and total DPD) was measured in a hydrolysed sample according to a modification of a previously published technique [27]. After the cross-links had been extracted by cellulose chromatography, they were recovered by reverse-phase HPLC at a flow rate of 1 ml/min using isocratic elution with 10% acetonitrile in 0.15% HBFA, and quantified by fluorimetry. Urinary excretion of Glc-Gal-PYD was measured by the same technique without the initial hydrolysis step and with isocratic elution using 6.5% acetonitrile in 0.15% HBFA. The amount of Glc-Gal-PYD was quantified using purified bone PYD, which was also used to quantify urinary PYD, as the standard [27].

All data obtained from urinary assays were corrected by the urinary creatinine concentration measured by a Jaffe colorimetric method using a kit from Konelab (Espoo, Finland).

Statistical analysis

Comparison of the markers between controls, patients with RA and Paget’s disease was assessed with the non-parametric Mann–Whitney U-test. The effect of

![Image](image-url)

**Fig. 1.** Typical relative fluorescence chromatograms of (a) fractionated urine from a patient with RA, (b) Glc-Gal-PYD standard purified from children’s urine, and (c) Glc-Gal-PYD standard after acid hydrolysis. (d) UV trace (monitored at 280 nm) of the Glc-Gal-PYD standard shown in (b). Samples were extracted and analysed by reverse-phase HPLC as described in the text.
bisphosphonate on the markers was evaluated with the Wilcoxon rank sum test. Correlation between urinary excretion of Glc-Gal-PYD, CRP and ESR was assessed by Spearman correlation analysis.

Results

Identification and characterization of urinary Glc-Gal-PYD

A typical chromatogram of urine from a patient with RA revealed that Glc-Gal-PYD was eluted before PYD and DPD (Fig. 1a). A linear gradient of 6.5% of acetonitrile allowed good separation between the different peaks, with an elution time of 13.5–14.5, 18.5–19.5 and 24.5–25.5 min for Glc-Gal-PYD, PYD and DPD respectively. Approximately 1 mg of Glc-Gal-PYD was purified from 50 l of urine (Fig. 1b). Acid hydrolysis of Glc-Gal-PYD gave a peak which co-eluted with PYD (Fig. 1c). To verify the purity of Glc-Gal-PYD, the isolated standard was also monitored with a UV detector at 280 nm, and no significant contamination was observed (Fig. 1d). Mass spectrometry of the Glc-Gal-PYD standard showed a major peak with a molecular mass of 752.2 (corresponding to the mass of Glc-Gal-PYD minus one hydrogen mass), in agreement with the theoretical mass of Glc-Gal-PYD reported previously [28] (Fig. 2).

Analysis of pyridinium cross-links in tissue after acid and alkaline hydrolysis

HPLC chromatography of synovium and cartilage alkaline hydrolysates (Fig. 3a) showed a Glc-Gal-PYD compound which co-eluted with the Glc-Gal-PYD urinary standard (Fig. 3b) and was transformed into PYD after acid hydrolysis (data not shown). Chromatography of bone alkaline hydrolysate (Fig. 3a) showed a compound (labelled X in Fig. 3) which did not co-elute with the Glc-Gal-PYD standard (Fig. 3b), but was transformed into PYD after acid hydrolysis (data not shown). The compound corresponding to peak X is likely to be the monoglycosylated form of PYD, galactosyl PYD (Gal-PYD). The use of a linear gradient of 5.5% acetonitrile allowed the best partition of Glc-Gal-PYD and Gal-PYD, which were eluted at 15–17 and 17–18 min respectively.

Total PYD (PYD + Glc-Gal-PYD + Gal-PYD), total DPD and Glc-Gal-PYD are expressed per gram of dry tissue in Table 1. Cartilage and synovium had the highest PYD content (respectively 13 and 8 times higher than in bone). Muscle and liver had a low PYD content. The hydrolysis of tissues by sodium hydroxide after 5, 10, 15 and 20 h resulted in the release of about 10% of the total PYD, as determined after total digestion of tissue for 20 h in 6 M hydrochloric acid, with very similar results for all five tissues. For Glc-Gal-PYD, values were obtained by multiplying the raw data for

Fig. 2. Negative-ion electrospray mass spectrum of Glc-Gal-PYD. Formula C96H49O18N4, theoretical mass 753.5. *Peak (752.2) corresponding to the measured mass of Glc-Gal-PYD minus one hydrogen mass. **Peak (774.2) corresponding to the measured mass of Glc-Gal-PYD plus one sodium mass minus two hydrogen masses. ***Peak (796.1) corresponding to the measured mass of Glc-Gal-PYD plus two sodium masses minus three hydrogen masses.
alkaline hydrolysis by a factor that took into account the yield of alkaline hydrolysis. Glc-Gal-PYD was particularly abundant in synovium, where the content was 10- to 20-fold higher than in cartilage, muscle and liver. Glc-Gal-PYD was not detectable in bone.

It should be noted that after 5 h of alkaline hydrolysis, the Glc-Gal-PYD content was close to that of PYD in synovium: the Glc-Gal-PYD : PYD ratio was 1:1, whereas it was only 0.05:1 for cartilage. With increasing time of alkaline hydrolysis, the tissue concentration of Glc-Gal-PYD decreased; the Glc-Gal-PYD : PYD ratio at 20 h of alkaline hydrolysis was 0.35 : 1 for synovium and 0.03 : 1 for cartilage. In bone the Gal-PYD : PYD ratio was 0.58 : 1 at 5 h of alkaline hydrolysis and 0.36 : 1 at 20 h.

**Glc-Gal-PYD is released during the degradation of synovium tissue in culture**

In the supernatant of synovium tissue culture, Glc-Gal-PYD represented 25-60% of the PYD content depending on the culture medium (Fig. 4a). Glc-Gal-PYD seemed to be present in the supernatant of cartilage tissue, but only in minute amounts (Fig. 4b), and was not found in the supernatant of bone tissue (Fig. 4c).
Table 1. Total PYD, total DPD and Glc-Gal-PYD in human tissues (nmol/g dry tissue; mean ± S.E.M.)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>n</th>
<th>Age (yr)</th>
<th>Total PYDa</th>
<th>Total DPD</th>
<th>5b</th>
<th>10b</th>
<th>15b</th>
<th>20b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synovium</td>
<td>6</td>
<td>63 ± 11</td>
<td>490 ± 20</td>
<td>9 ± 2</td>
<td>218 ± 14</td>
<td>180 ± 22</td>
<td>164 ± 30</td>
<td>116 ± 44</td>
</tr>
<tr>
<td>Bone</td>
<td>4</td>
<td>57 ± 8</td>
<td>59 ± 5</td>
<td>16 ± 4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cartilage</td>
<td>4</td>
<td>57 ± 8</td>
<td>783 ± 65</td>
<td>18 ± 3</td>
<td>35 ± 15</td>
<td>43 ± 35</td>
<td>40 ± 38</td>
<td>23 ± 23</td>
</tr>
<tr>
<td>Muscle</td>
<td>2</td>
<td>59 ± 8</td>
<td>28 ± 10</td>
<td>5 ± 2</td>
<td>9 ± 5</td>
<td>8 ± 4</td>
<td>7 ± 2</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Liver</td>
<td>2</td>
<td>59 ± 8</td>
<td>25 ± 3</td>
<td>3 ± 1</td>
<td>9 ± 1</td>
<td>10 ± 1</td>
<td>5 ± 1</td>
<td>3 ± 1</td>
</tr>
</tbody>
</table>

Total PYD = (PYD + Glc-Gal-PYD + Gal-PYD).

Analytical performance of HPLC assay of Glc-Gal-PYD

The reproducibility of the urinary assay was assessed by eight repeated measurements of urine samples. The intra- and interassay variation was less than 13 and 12% respectively. Analytical recovery, assessed by adding Glc-Gal-PYD standard to different urine samples, averaged 96%. Dilution of urine with a high initial concentration of Glc-Gal-PYD was tested (1/2, 1/5 and 1/10), and the overall recovery was 102%. Five repeated freeze-thaw cycles did not substantially alter the urine concentration in the two samples tested, with a mean recovery of 96 ± 7%. The stability of Glc-Gal-PYD was tested on two urine samples kept in the dark at room temperature or at 4°C for 10 days. The urinary concentration of Glc-Gal-PYD was stable for 3 and 5 days at room temperature and 4°C respectively. After 10 days, the urinary concentration of Glc-Gal-PYD had decreased by 37 and 26% at room temperature and 4°C respectively.

Urinary excretion of Glc-Gal-PYD in normal adults

Values for urinary total PYD, total DPD and Glc-Gal-PYD were measured in 69 normal adults classified according to age and sex (Table 2). Total PYD and total DPD increased significantly with age. In contrast, there was no statistically significant change in Glc-Gal-PYD with age.

Urinary excretion of Glc-Gal-PYD in patients with RA and Paget’s disease of bone

There was a significant increase in the mean levels of total PYD and total DPD in patients with Paget’s disease and in patients with RA compared with controls, with a larger increase in PYD in RA and in DPD in Paget’s disease (Table 3 and Fig. 5). Interestingly, mean values of Glc-Gal-PYD were significantly increased in patients with RA but not in patients with Paget’s disease compared with controls (Fig. 5). After i.v. bisphosphonate treatment of patients with Paget’s disease, total PYD and DPD were significantly decreased, by 25 and 33%, respectively, contrasting with the absence of change in Glc-Gal-PYD.

The Glc-Gal-PYD peak in synovium and cartilage media co-eluted with the standard purified from human urine. In synovium medium, Glc-Gal-PYD peaks were collected and hydrolysed by 6 N hydrochloric acid to verify that they were transformed into PYD (data not shown).
Table 2. Urinary excretion of total PYD, total DPD and Glc-Gal-PYD in healthy adults

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Age (yr)</th>
<th>Total PYD (nmol/mmol Cr)</th>
<th>Total DPD (nmol/mmol Cr)</th>
<th>Glc-Gal-PYD (nmol/mmol Cr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premenopausal</td>
<td>20</td>
<td>35.7 ± 2.8</td>
<td>28.5 ± 4.1</td>
<td>5.9 ± 1.4</td>
<td>4.5 ± 1.0</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>19</td>
<td>55.2 ± 2.5</td>
<td>34.0 ± 8.6*</td>
<td>7.6 ± 2.7</td>
<td>4.5 ± 1.1</td>
</tr>
<tr>
<td>Elderly women</td>
<td>16</td>
<td>69.1 ± 5.0</td>
<td>49.3 ± 14.8**</td>
<td>12.4 ± 5.2**</td>
<td>4.6 ± 1.5</td>
</tr>
<tr>
<td>Men</td>
<td>14</td>
<td>59.4 ± 7.4</td>
<td>33.1 ± 10.9</td>
<td>6.5 ± 2.7</td>
<td>4.4 ± 1.8</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.001 compared with premenopausal women (Mann–Whitney U-test).

Table 3. Urinary excretion of total PYD, total DPD and Glc-Gal-PYD in patients with RA and Paget’s disease

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Age (yr)</th>
<th>Total PYD (nmol/mmol Cr)</th>
<th>Total DPD (nmol/mmol Cr)</th>
<th>Glc-Gal-PYD (nmol/mmol Cr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>35</td>
<td>55.8 ± 7.1</td>
<td>33.3 ± 9.3</td>
<td>7.0 ± 2.7</td>
<td>4.6 ± 1.4</td>
</tr>
<tr>
<td>RA</td>
<td>27</td>
<td>56.2 ± 11.9</td>
<td>72.2 ± 51.8**</td>
<td>10.8 ± 7.6*</td>
<td>9.6 ± 5.9**</td>
</tr>
<tr>
<td>Paget’s disease</td>
<td>13</td>
<td>69.0 ± 9.8</td>
<td>98.0 ± 45.9**</td>
<td>27.3 ± 18.8**</td>
<td>5.4 ± 1.5</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.001 compared with controls (Mann–Whitney U-test).

% increase over controls

![Graph showing % increase over controls](image)

Fig. 5. Increased urinary levels of total PYD, total DPD and Glc-Gal-PYD in patients with Paget’s disease and RA. Bars represent mean (± S.E.M.) percentage increases in the markers over the mean for healthy controls. Statistical significance (Mann-Whitney U-test): *P < 0.05, **P < 0.001 compared with controls.

Correlation between urinary excretion of Glc-Gal-PYD, inflammation markers and joint erosion score

Significant positive correlations were noted between urinary Glc-Gal-PYD, CRP and ESR (Table 4). When RA patients were divided into those with and without destructive RA, urinary excretion of Glc-Gal-PYD was significantly increased in those patients with destructive disease (12.2 ± 7.4 and 7.5 ± 3.2 nmol/mmol creatinine in destructive RA and non-destructive RA respectively; P < 0.05). Significant correlations were obtained between Glc-Gal-PYD and the number of joints involved. No such correlations were noted for non-destructive RA patients (Table 4).

Discussion

In this study we describe a post-translational modification of collagen, the diglycosylation of PYD, which appears to be specific for synovium tissue. We also show that Glc-Gal-PYD is released during the degradation of synovium in culture. The urinary excretion of Glc-Gal-PYD is increased in patients with RA and is related to the extent of joint destruction.

We found that Glc-Gal-PYD was abundant in synovium tissue, present in minute amounts in cartilage and absent in bone. This indicates strongly that the glycosylation of PYD differs between joint tissues and that the diglycosylated form represents a collagen cross-link specific to synovium. PYD has been shown previously to be present in other connective tissues and especially in skeletal muscle, though in lower amounts [13, 14, 24, 29, 30]. In this study, we found also that Glc-Gal-PYD was present in minute amounts in muscle and liver, suggesting that this cross-link discriminates not only between bone, cartilage and synovium, but probably also between synovium and other non-joint soft tissues. However, a complete analysis of all human tissues would be required to establish the specificity of this cross-link. The yield of alkaline hydrolysis is only 10% of that of acid hydrolysis and the amount of Glc-Gal-PYD as a percentage of PYD decreases with increasing hydrolysis time, presumably because of the partially alkaline lability of glycosylation [31]. Thus, our data for Glc-Gal-PYD in the tissues are likely to give only an approximation of the content of Glc-Gal-PYD. Because the yield was similar for all tissues, comparison between bone, cartilage and synovium is likely to be valid.

Table 4. Correlation coefficients between Glc-Gal-PYD and CRP, ESR and joint erosion score

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>CRP</th>
<th>ESR</th>
<th>Number of diseased joints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole RA population</td>
<td>27</td>
<td>0.47**</td>
<td>0.45**</td>
<td>0.34</td>
</tr>
<tr>
<td>Destructive RA</td>
<td>12</td>
<td>0.30**</td>
<td>0.45**</td>
<td>0.66*</td>
</tr>
<tr>
<td>Non-destructive RA</td>
<td>15</td>
<td>0.62*</td>
<td>0.40</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*P < 0.001, **P < 0.05.
In the supernatant of human synovium tissue culture, the ratio Glc-Gal-PYD : PYD was similar to that in the alkaline hydrolysate of the corresponding tissue, suggesting that this glycosylated cross-link reflects adequately the rate of synovium turnover. Glc-Gal-PYD was found only in minute amounts in the supernatant of cartilage culture and was not detectable in the supernatant of bone, in agreement with the tissue analysis. Altogether, our data indicate that Glc-Gal-PYD is almost specific for synovium tissue and that it is released during the process of synovium tissue turnover.

To confirm the potential clinical value of Glc-Gal-PYD, we measured its urinary excretion in normal adults, patients with Paget’s disease (a bone-specific disease) and patients with RA. We found that urinary Glc-Gal-PYD did not increase after the menopause or in patients with Paget’s disease, and remained unchanged after treatment with bisphosphonate, which specifically inhibits bone resorption. These results contrast with the increase in urinary total PYD and total DPD in postmenopausal women and in Paget’s disease, resulting from the increase in bone turnover [32]. Thus, our in vivo data indicate that urinary Glc-Gal-PYD does not reflect changes in bone turnover, in agreement with our initial results. In addition, urinary excretion of Glc-Gal-PYD appears to be independent of sex and age in the range tested, although a larger sample of individuals needs to be tested to assess this in detail.

In patients with RA, we found a larger increase in total PYD compared with total DPD (117 vs 55%), resulting in a urinary PYD : DPD ratio of 6.7:1, in agreement with previous studies [17–19]. Although this ratio is higher than that in urine from patients with Paget’s disease and higher than that in bone matrix (3.6 : 1), it is much lower than those for cartilage and synovium tissue (45 : 1 and 27 : 1 respectively), suggesting that in RA bone turnover accounts for the largest part of urinary PYD excretion. In patients with RA, there was a twofold increase in urinary Glc-Gal-PYD, and levels were correlated moderately with indices of inflammation such as CRP and ESR. However, these CRP and ESR levels explained only 20% of the interindividual variability of Glc-Gal-PYD, clearly indicating that Glc-Gal-PYD does not reflect only the inflammation process. Interestingly, in patients with RA, Glc-Gal-PYD was more elevated in those with joint destruction than in those with non-destructive disease, and levels correlated with the number of joints involved in the disease. These data indicate that, in addition to reflecting synovial tissue inflammation, urinary Glc-Gal-PYD is also potentially useful in the assessment of the process of destruction, although larger prospective studies are required to confirm this hypothesis.

In conclusion, we have characterized a new post-translational modification of collagen which appears to be highly abundant in synovium tissue, present in minute amounts in cartilage and absent from bone. The urinary excretion of Glc-Gal-PYD increases in patients with RA, but not in patients with Paget’s disease, confirming in vivo its tissue specificity. This new marker may be useful in the clinical investigation of patients with RA and OA.

References