Concentrations of pyridinoline and deoxypyridinoline in joint tissues from patients with osteoarthritis or rheumatoid arthritis

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Abstract

Objective—To assess the usefulness of pyridinoline (Pyr) and deoxypyridinoline (Dpyr), intermolecular crosslinks of collagen, as markers in the evaluation of arthritis, by studying their distribution in tissues from knee joints.

Methods—Joint tissues (cartilage, bone, synovium) were obtained during operation from 10 patients with osteoarthritis (OA) and 10 patients with rheumatoid arthritis (RA). Synovium was also obtained from 10 non-arthritic (NA) subjects. Hydroxyproline was measured in hydrolysed tissue samples and converted to an equivalent collagen content. The amounts of Pyr and Dpyr crosslinks measured in the hydrolysed samples using a fluorescence technique were expressed as $\mu\text{mol/mol}$ of collagen.

Results—Pyr and Dpyr were distributed in all three tissues, but in different amounts. The ratio of the contents of Pyr and (Pyr:Dpyr) was 50:1 in cartilage, 3:1 in bone, and 25:1 in synovium. OA cartilage had a greater Dpyr content than the RA cartilage, but there was no other significant difference in the contents of Pyr and Dpyr and the ratio Pyr:Dpyr in the joint tissues from patients with OA or RA. In synovium, there was no significant difference between the contents of Pyr and Dpyr and the Pyr:Dpyr ratio among OA, RA, and NA tissues.

Conclusion—Both Pyr and Dpyr were located in cartilage, bone, and synovium. A significant amount of Pyr and Dpyr in these joint tissues, especially in synovium, may contribute to the urinary excretion of those crosslinks that is observed in arthritis.

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Pyridinoline (Pyr),^{1,2} and its analogue, deoxypyridinoline (Dpyr),³ are non-reducible crosslinks of mature collagen. The distribution of the pyridinium crosslinks was reported a decade ago: Pyr is distributed in most collagenous tissues, primarily in cartilage and bone,⁴ while significant amounts of Dpyr were reported to be distributed only in bone and dentin. However, as Dpyr has subsequently been detected in cardiovascular muscle, aorta,

and cartilage in disease states,^{5,6} its distribution is evidently not restricted to bone and dentin. The tissue distribution of pyridinium crosslinks thus requires reassessment.

The urinary concentrations of Pyr and Dpyr have been used as markers of bone metabolism, especially bone resorption, and many observers have reported the usefulness of these urinary Pyr and Dpyr values in the assessment of various metabolic bone diseases.⁷ In contrast, as the tissue distribution of Pyr favours cartilage, in addition to its presence in bone, urinary Pyr content may serve as a marker of cartilage metabolism. High urinary excretion of Pyr has been observed in a preliminary analysis in osteoarthritis (OA)⁸ and in rheumatoid arthritis (RA),⁹ and several observers have proposed that urinary pyridinium crosslinks could be markers of arthritis.^{10–15} However, relationships between the distribution of pyridinium crosslinks in the joint tissues, the origin of urinary Pyr or Dpyr crosslinks, and the significance of the urinary excretion of the crosslinks as biochemical markers in arthritis, have not been established. The purpose of this study was to examine the distributions of two crosslinks, Pyr and Dpyr, in joint tissues, in order to assess the potential usefulness of urinary Pyr and Dpyr as biochemical markers for the evaluation of arthritis.

Patients and methods

Articular cartilage, subchondral cancellous bone, and synovial tissue were obtained during operations involving total knee replacement, from 10 patients with OA (aged 58–79 years) and 10 patients with RA (aged 44–73 years), in the Department of Orthopaedic Surgery at the Hamamatsu University School of Medicine. OA patients were diagnosed as having osteoarthritic knee joints on the basis of clinical symptoms, examination, and radiological findings; RA patients were diagnosed according to the American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis.¹⁶

Synovium was also obtained arthroscopically from 10 non-arthritic (NA) patients (aged 13–76 years) who had suffered trauma to the knee joints. The procedures followed were in accordance with the principles of the 1975 Declaration of Helsinki, as revised in 1983.

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MEASUREMENT OF CROSSLINKS IN TISSUES

The crosslink content of the tissues was measured by a modification of a method described previously.¹⁷ Briefly, cancellous bone was powdered in liquid nitrogen and demineralised repeatedly with 0.5 mol/l EDTA, pH 7.4 (four changes of EDTA solution), cartilage was dissected and homogenised with a Polytron homogeniser, and synovial tissue was dissected and minced. Dissected cartilage and synovium samples were defatted and cleaned by washing with acetone and saline. Samples were hydrolysed in 6 mol/l hydrochloric acid (HCl) (10 mg wet weight/ml) at 110°C for 20 hours in a sealed glass tube. The hydrolysate (0.125 ml) was mixed with 15 ml of distilled water and applied to an SP-Sephadex C25 column (0.8 × 1.0 cm). After washing with 20 ml of 0.15 mol/l HCl, Pyr and Dpyr were eluted with 5 ml of 1.0 mol/l HCl. After evaporation, the residue was dissolved in 200 µl of a 1% heptafluorobutyric acid (HFBA) solution. The solutions were stored at -30°C until required for analysis by high performance liquid chromatography (HPLC). The HPLC system consisted of a pump (Model CCPM, TOSOH, Tokyo, Japan), a spectrofluorimeter (Model FS-8010, TOSOH), and a system controller (Model SC-8010, TOSOH). A column (8 mm × 10 cm) prepacked with Radial-Pak C18, of 10 µm particle size, type 8C1810u (Waters Associates Inc, Milford, MA, USA) was used. A mobile

phase of acetonitrile and 40 mmol/l HFBA (27:73, v/v) was used, with a flow rate of 1.0 ml/min. The volume of each sample injected was 160 µl. The fluorescence at 390 nm was measured on excitation at 297 nm. The lower limit of detection of Pyr (signal to noise ratio = 4) was 1.2 pmol per one injection under our experimental conditions.

The hydroxyproline content of the hydrolysed tissue samples was measured on a Model 835-50 automated amino acid analyser system (Hitachi, Tokyo, Japan).¹⁸ The collagen content was calculated assuming 285 mol of hydroxyproline/mol of collagen, and the amounts of crosslinks contained in the samples were expressed per mol of collagen.

STATISTICAL ANALYSIS

The statistical significance of differences was determined with non-parametric statistics using Mann-Whitney *U* tests between two groups and by Kruskal-Wallis among three groups. The analysis was performed with StatView II on a Macintosh computer. Values of *p* less than 0.05 were considered significant.

Results

Figure 1 shows typical chromatograms of the joint tissues from patients with OA or RA, and of synovium from non-arthritic subjects. The chromatogram profile of each tissue was

Figure 1 Typical chromatograms of joint tissues (cartilage, bone, and synovium) in osteoarthritis (OA) and rheumatoid arthritis (RA), and synovium from non-arthritic joints (NA). ▼ = Pyridinoline; ▽ = deoxypyridinoline.

Concentrations of pyridinoline (Pyr) and deoxypyridinoline (Dpyr) and their ratio (Pyr:Dpyr) in joint tissues of patients with osteoarthritis (OA) or rheumatoid arthritis (RA)

		Pyr* (mmol/mol collagen)	Dpyr (mmol/mol collagen)	Pyr:Dpyr*
Cartilage	OA	988 (86)	26.6 (2.6)†	41.2 (6.5)
	RA	1047 (121)	19.0 (2.5)	67.7 (12.5)
Bone	OA	139 (16)	46.9 (4.7)**	3.0 (0.2)
	RA	157 (16)	46.8 (5.6)**	3.5 (0.2)
Synovium	OA	466 (35)	25.6 (4.2)	25.6 (6.2)
	RA	485 (26)	19.4 (2.6)	30.4 (5.0)

Values are mean (SE). Pyr:Dpyr was calculated for each sample; the means (SE) of these are presented in the last column.
†Significant difference ($p < 0.05$) compared with value for RA (Mann-Whitney U test).
*Significant differences ($p < 0.05$) among cartilage, bone, and synovium from both OA and RA (Kruskal-Wallis). **Significant difference ($p < 0.01$) compared with values in cartilage and synovium (Kruskal-Wallis).

similar in OA and RA, and the chromatogram profile of synovial tissue was similar among OA, RA, and NA tissues.

The table shows the concentrations of crosslinks and the Pyr:Dpyr ratio in cartilage, bone, and synovium from OA and RA joints. For each of the tissues there was no significant difference between OA and RA in the concentrations of crosslinks and the Pyr:Dpyr ratio, except that the concentration of Dpyr in OA cartilage was significantly greater than that in RA cartilage ($p < 0.05$). In both OA and RA, the concentrations of Pyr and the Pyr:Dpyr ratio were significantly different when the three joint tissues were compared ($p < 0.05$). The Pyr:Dpyr ratio in bone was approximately 3, that in synovial tissues was about 25, and that in cartilage was approximately 50. The concentrations of Dpyr in bone were significantly greater than those in cartilage and synovium ($p < 0.01$), but there was no significant difference in Dpyr content between cartilage and synovium.

Figure 2 shows the concentrations of Pyr and Dpyr, and their ratio, in synovium from OA, RA, and non-arthritis (NA) subjects. The Pyr:Dpyr ratio tended to be greater in RA than in samples from non-arthritis subjects, but there was no significant difference in the content of Pyr and Dpyr, and the Pyr:Dpyr ratio among the OA, RA, and NA groups.

Discussion

Although Pyr and Dpyr are both mature crosslinks of collagen molecules in the tissues, their distributions and concentrations in various tissues are different. Pyr is most

abundant in bone and cartilage, though it is widely distributed in most tissues except the skin. Urinary Pyr is generally considered to have its origin in bone, as the turnover of bone is believed to be much greater than that of the other tissues,⁷ and because the distribution of Pyr among the tissues favours cartilage and bone, urinary Pyr crosslinks have been proposed as markers of arthritis. It has recently been suggested¹⁹ that these crosslinks in urine might originate primarily from periarticular lesions of bone in OA, and as osteopenia is known also to be a major pathology in RA, the increased urinary concentrations of these crosslinks in RA may also originate partly from periarticular lesions of bone, in addition to bone itself.

In contrast to the wide distribution of Pyr, that of Dpyr has been considered to be more restricted—primarily to bone and dentin—and it was therefore proposed that Dpyr could be a more specific marker of bone metabolism than Pyr. However, the distribution of Dpyr in ligament and aorta has subsequently been reported,^{20, 21} and our measurements of the concentrations of crosslinks in joint tissues in the present study have shown that Dpyr was also located in cartilage and synovium. Although no data have been published previously concerning the synovial content of Pyr and Dpyr, in this study we have found a significant amount of both in synovium. As synovial tissues undergo relatively rapid turnover in arthritis, especially in RA, the urinary excretion of Pyr and Dpyr may correlate with the turnover and inflammation of synovium.

We reported previously that the joint disorder did not affect the content of Pyr in cartilage.¹⁷ In the present study, the type of arthritis did not affect the content of pyridinium crosslinks in the joint tissues, while in synovium, the content of pyridinium crosslinks did not change in arthritic compared with non-arthritic tissues. Changes in urinary excretion of pyridium crosslinks therefore reflect the turnover of joint tissues affected by arthritis, but not change in the content of crosslinks in arthritic tissues.

Although Dpyr was found in both cartilage and synovium, its proportion in relation to Pyr is much less in all other tissues (including cartilage and synovium) than in bone. Several observers have measured urinary pyridinium crosslinks in arthritis: most previous studies examining OA or RA reported a tissue Pyr:Dpyr ratio of 4–5, though the ratio excreted in the urine ranged from 3.3 to 11.4. Although no tendency of change in the ratio of Pyr to Dpyr in arthritis has been established in previous published data, one study showed that the ratio was greater in RA than in control patients,¹⁰ and it has also been reported that the ratio was greater in active RA than in inactive RA.¹¹ As the results of our study have shown the ratio of Pyr to Dpyr to be much greater in cartilage (50:1) and synovium (25:1) than in bone (3:1), it is likely that part of the urinary excretion of Pyr and Dpyr may be contributed from tissues other than bone,

Figure 2 Concentrations of pyridinoline (Pyr) and deoxypyridinoline (Dpyr) and their ratio (Pyr:Dpyr) in synovium from patients with osteoarthritis (OA) or rheumatoid arthritis (RA), and non-arthritis controls (NA). Bar indicates SE.

namely cartilage and synovium. The urinary concentrations of Pyr and Dpyr and the Pyr:Dpyr ratio are dependent on two factors: the relative abundance of the crosslinks in the tissues and the rate of turnover of the tissues. As cartilage and synovium have a much greater Pyr:Dpyr ratio than has bone, increased turnover of cartilage or synovium, or both, would contribute little to urinary excretion of Dpyr, but would significantly increase urinary excretion of Pyr. If the turnover rate of cartilage and synovium is the same, urinary excretion of Pyr would receive a greater contribution from cartilage than from synovium, because of the greater Pyr content and greater Pyr:Dpyr ratio of cartilage. However, if the rate of turnover of synovium is greater than that of cartilage, synovium would contribute more to the urinary excretion of Pyr than would cartilage.

In conclusion, cartilage and synovium contribute to the urinary excretion of pyridinium crosslinks, and the crosslinks may thus be useful as indices of arthritis. Moreover, the significant content of pyridinoline found in synovium suggests that the urinary excretion of pyridinoline may also reflect synovial turnover and synovial inflammation.

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