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Tissue Proline Imino-Peptidase Activity in Osteoarthritis, Rheumatoid Arthritis and Dupuytren's Contracture

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=국문초록=

退行性関節炎, 류마치스性関節炎과 드퓌트렌拘縮症의 組織內 Proline Imino-Peptidase 活性度

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膠原質의 대사량은 24시간뇨중의 하이드록시-프로린 양(THP)을 측정함으로써 비교적 정확하게 알 수 있다. 그러나 이 방법은 食餌를 엄격히 조절하고 24시간뇨를 수집해야 하는 번거로운 절차때문에 보편적으로 이용되지 못하고 있다.

혈액과 결체조직에 존재하는 프로린 이미노-펩티데이즈(PIP)는 이러한 단점을 가진 THP측정방법을 대신할 수 있을 것으로 여겨진다. 교원질의 3重螺線状 구조 자체를 공격하는 교원질 분해효소는 아니지만, PIP는 생리적 및 실험적 조건에서 교원질 분해효소와 비례하는 活性度를 보여주기 때문에 체내의 교원질 대사량을 정확히 알려주는 지수역할을 해낼 수 있고, 또 合成基質을 사용 시험관내에서 쉽게 측정되기 때문이다.

저자는 퇴행성 관절염 38예, 류마치스성 관절염 26예로부터 얻어진 관절조직과 드퓌트렌 구축증 5예로부터 얻어진 증식조직에 대하여 조직내 PIP 활성도를 측정하여 질환에 따른 교원질 대사의 양적 차이를 대조하고, 동시에 조직내 PIP측정의 효용성을 시험하여 긍정적인 결과를 얻을 수 있었다. 이에 그 결과를 문헌고찰과 함께 보고하는 바이다.

INTRODUCTION

The most abundant protein in human body is collagen, the fibrous component of the several varieties of connective tissues. It is well known that a fairly constant fraction of the peptide bound and free hydroxyproline released during its breakdown is excreted in the urine.[7,13] For this reason, and since this imino acid is derived almost exclusively from collagen, the total hydroxyproline excretion in the urine provides a useful indirect measure of collagen metabolism. This has been used particularly to indicate the turnover rate of bone matrix collagen in patients with bone disease.

However there are drawbacks in technique and, more importantly, in interpretation. Accurate measurement of total hydroxyproline requires control of diet, since its excretion is influenced by dietary intake of collagen.[9] The interpretation of its excretion depends on age and it is impossible to identify which collagenous tissue has mainly contributed to it.[10]

In vivo, collagen degradation is initiated by collagenase and possibly by other proteases.[1,2,6] The resulting peptides, which contain hydroxyproline, are further degraded, probably by proline iminopeptidase (PIP) which is present in the circulation as well as in the local tissue.[5,14] It is this enzyme which may provide an alternative index of collagen degradation.[15] We have measured its activity in synovia and capsules from two major joint diseases which are rheumatoid arthritis and osteoarthritis, and in tissues excised from Dupuytren's contracture in order to find the differences of pathological process being evolved locally from the view point of collagen breakdown.

Key words : Collagen breakdown, Tissue PIP activity, Arthritis.

MATERIALS AND METHODS

Tissues obtained at the time of surgery from the following three groups of patients are studied:

1. Osteoarthritis group (38 patients; 20 male, 18 female; age range 21-82 years, mean 64 years). The diagnosis was established clinically and confirmed post-operatively by histological examination of excised tissues.

2. Rheumatoid arthritis group (26 patients; 5 male, 21 female; age range 42-73 years, mean 61 years). All patients had "definite" rheumatoid arthritis (by A.R.A. criterion).

3. Dupuytren's contracture group (5 patients all male; age range 46-71 years, mean 61 years). The diagnosis was established clinically.

Synovial and where possible capsular tissues were obtained from involved joints in groups 1 and 2 (See Table 1) and proliferative tissue in palmar fascia in group 3.

Materials

The synthetic substrate, p-phenylazo-benzyloxy-carbonyl-Pro-Leu-Gly-Pro-D-Arg (PZ-pentapeptide) was obtained from the Fluka Chemical Company. All other reagents were of AR grade. A 1.0 mmol/l solution of the substrate was prepared by adding 40.6 mg of PZ-pentapeptide to 20μl of dimethylformamide, the volume then being made up to 50 ml. with barbitone buffer (50 mmol/l sodium barbitone, adjusted to pH 8.1 with 50 mmol/l HCl).

P.I.P. assay

The method is based on that of Gries and co-workers as adapted by Whitely et al (1976).

Tissue homogenate preparation: The tissue specimen was dissected with fine scissors to separate synovial from capsular tissue. The tissue prepared like this or as it was from Dupuytren's contracture was minced and divided into aliquots of about 0.25 gm. in weight. Five ml. of 0.01 M phosphate buffer (pH 7.0) was added and the suspension homogenized with an 'ultra turrex' homogenizer at minimum speed for 50 secs. The homogenate was centrifuged for 10 mins. at 900 x g and the supernatant removed and filtered. The residue was resuspended in a further 20 ml. of 0.01 M phosphate buffer (pH 7.0), centrifuged as above and the residue taken for PIP assay. One ml. of PZ-substrate was added to 0.5 ml. of filtered supernatant or to the total residue suspended in 0.5 ml. of 0.01 M phosphate buffer, pH 7.0. The mixture was then incubated for 4 hrs. at 37°C. after which the reaction was stopped by the addition of 0.5 ml. of 480 mmol/1 citric acid. Blanks were prepared by addition of citric acid before incubation. The PZ-dipeptide released from the PZ-pentapeptide was extracted into 5.0 ml. of ethylacetate by vigorous shaking for 1 min. After centrifugation for 10 mins. at 900 x g, the ehtylacetate layer was removed and mixed 3.0 ml. of 120 mM/l citric acid solution. After further centrifugation for 10 mins. at 900 x g, the ethylacetate layer was removed, kept at 27°C and its absorbance read at 320 nm.

One unit of enzyme activity is defined as that which released 1.0 mmol of the PZ-dipeptide from the PZ-pentapeptide per Kg. of tissue after incubation at 37°C and pH 8.1 (the conditions under which maximum activity was formed).

Statistical methods

Student's t-test was used.

RESULTS

P.I.P. assay

Preliminary experiments demonstrated that maximum activity was extracted if homogenization was carried for 50 seconds at minimum homogenizer speed at 4% W : V (tissue : medium) and that P.I.P activity was maximal at pH 7.8—8.6, proportional to the amount of tissue used (up to 1 gm) and the incubation time (up to 4 hrs). Activity was maintained during storage at -27°C but decreased if the samples were left overnight at room temperature. Activity was destroyed by heating at 100°C for 10 minutes.

Solubility and P.I.P. activity

P.I.P. activity in the tissue residue was consistently below about 10% of that in the supernatant fraction (Table 1). This proportion was constant in the varying tissues from the three groups of subjects. It was not possible to perform satisfactory blanks with the insolu-

Table 1. Tissue PIP activity according to different disease groups

Group	Tissue	n	Total Activity (M ± SE, μU/G)	Distribution of Activity	
				Soluble (% of Total)	Insoluble (% of Total)
Osteoarthritis	Synovium	38	10.4 ± 1.3	8.7 ± 1.5(84.5)	1.6 ± 0.3(15.4)
	Capsule	29	12.8 ± 1.4	10.4 ± 1.9(81.3)	2.4 ± 0.3(18.7)
Rheumatoid arthritis	Synovium	26	42.0 ± 3.3	38.2 ± 4.7(91.2)	3.7 ± 0.4(8.8)
	Capsule	18	16.6 ± 1.4	13.0 ± 2.6(78.3)	3.6 ± 0.9(21.7)
Dupuytren's Contracture	Palmar fascia	5	26.1 ± 7.4	22.9 ± 10.2(87.7)	3.2 ± 0.6(12.3)

Table 2. Differences in synovial PIP activity between sex and source of joint

Group	Sex		Source of tissue		
	Male	Female	Hip	Knee	Hand
Osteoarthritis	9.1 ± 1.9* (n=20)	8.3 ± 1.9 (n=18)	9.7 ± 1.9 (n=29)	6.2 ± 2.7 (n=8)	0 (n=1)
Rheumatoid arthritis	35.6 ± 13.8 (n=5)	38.9 ± 5.0 (n=21)	38.2 ± 5.2 (n=7)	38.1 ± 7.2 (n=10)	38.5 ± 8.7 (n=9)

* M ± SE, μU/g

ble fraction and the value obtained is therefore a maximum. Our results suggest therefore that most, if not all, the P.I.P. activity in these tissues is easily solubilized. Attempts to decrease the proportion of activity in the residue fraction of incubation of tissue with deoxycholate or heating/thawing were unsuccessful.

P.I.P activity in synovial tissues

Mean P.I.P. activity in synovia from osteoarthritic joints was 8.7 ± 1.5 μU/gm (mean ± S.E.) and from rheumatoid arthritic joints 38.2 ± 4.7 μU/gm ($P<0.01$, Figure 1). The range in osteoarthritic synovium was 0—27.9 μU/gm and in rheumatoid arthritic synovium was 6.2—96.9 μU/gm with 16/26 (62%) above the maximum value found for osteoarthritic synovium. No differences were found according to the source of tissue or sex (Table 2).

P.I.P. activity in synovial tissues from patients with rheumatoid arthritis was significantly higher than that from the surrounding capsular tissues but was similar in the two tissues from osteoarthritic patients (Fig. 1).

In the group with rheumatoid arthritis, there was no relationship with E.S.R. values (Fig. 2), nor between patients with and without rheumatoid factor (Rh-factor positive group 36.4 ± 5.7 μU/gm, n=19,

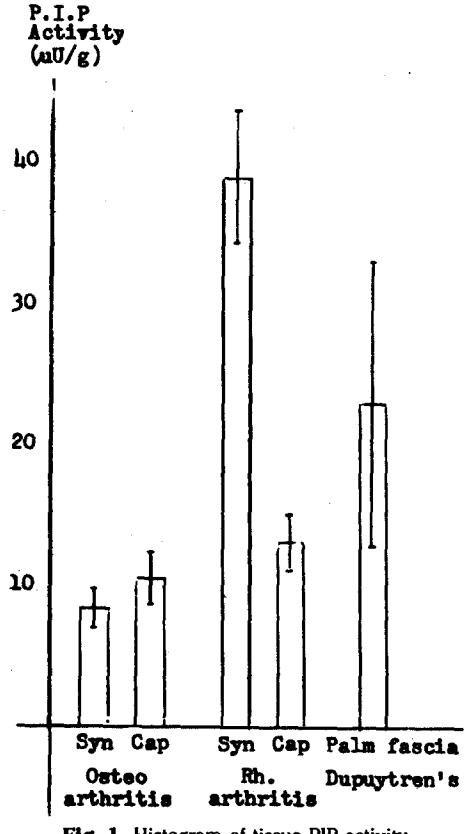

Fig. 1 Histogram of tissue PIP activity.

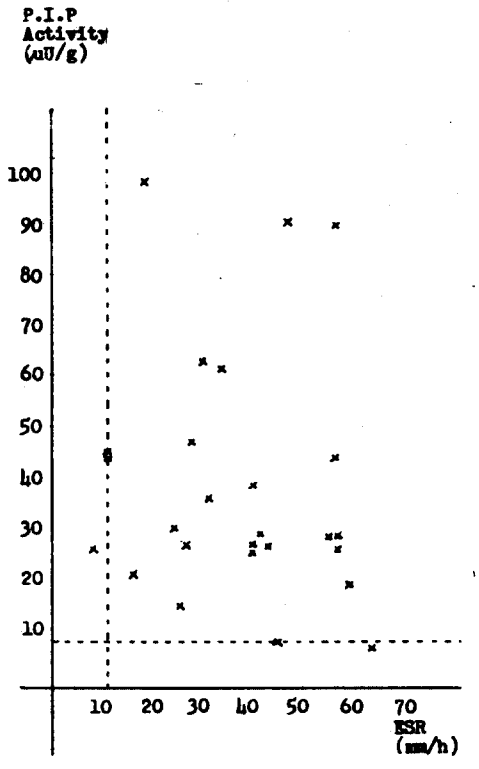

Fig. 2 Distribution of synovial PIP activity according to ESR. ——: Mean value of osteartritis group.

Table 3. Synopial PIP activity in different drug groups of Rheumatoid arthritis

N.S.A.I.D.*	Indomethacin	Prednisolone
44.2 ± 7.8** (n=8)	37.3 ± 6.1 (n=6)	29.2 ± 8.2 (n=9)

*N.S.A.I.D. = Non-Steroid Anti-Inflammatory Drug
**M ± SE, μU/g

negative group 43.20 uU/gm ± 11.2, n=7).

Patients with rheumatoid arthritis were divided into three groups according to the medical treatment they were receiving: (i) Indomethacin, (ii) Steroids and (iii) Other non-steroidal anti-inflammatory drugs. All patients in these groups had been receiving the drugs for at least three months prior to surgery. Results are given in Table 3. Synovial P.I.P activity was 96.9 and 39.8 μU/gm in two patients receiving D-penicillamine in addition to Prednisolone.

P.I.P. activity in capsular tissue

There were no significant differences in the mean P.I.P. activities between tissues from osteoarthritis and rheumatoid arthritis patients (Fig. 1), though the latter was higher. This could have resulted from tissue contamination of the capsular tissue with very active rheumatoid synovium as complete dissection was dificult.

P.I.P. activity in palmar fascia from Dupuytren's contracture

Mean P.I.P. activity in the soluble fraction of these tissues was 22.9 ± 10.2 μU/gm. This was significantly above the mean value for the soluble fraction from the synovial tissues from the osteoarthritis group ($P<0.05$) and lower, but not significantly, than that for the rheumatoid arthritis group. It was also higher than those from the capsular tissues (osteoarthritis group $P<0.01$, rheumatoid arthritis group $P<0.01$).

DISCUSSION

Proline imino-peptidase (PIP) is present in blood and connective tissues.[12,14,15] It is detected by its ability to hydrolyse the synthetic benzylazo-pentapeptide developed for the assay of bacterial collagenase.[12,15] In fact PIP does not split this peptide at the proline link, nor is a true collagenase as it does not cleave the triple helical collagen mollecule. Recent works suggested rather that it may act on relatively small peptides, some thirty amino-acid residues long, that result from the actions of collagenases, gelatinases and cathepsins on collagen.[12]

The levels of PIP seem to parallel those of collagenase under various physiological and experimental conditions. Its assay may thus be an alternative to that for collagenase as an index of tissue collagen degradation, especially as the assay is simple to perform.[4,5,12,14,15]

The most prominent and significant observation from the result of present work is marked elevation of PIP activity in synovial tissue from rheumatoid arthritis over from osteoarthritis. The wide difference between these two representative joint diseases with different pathogenesis provides sound ground for

some speculations.

First of all, it is confirmed that enzymatic degradation of local tissue is far more active in rheumatoid joint than in osteoarthritic joint as one may expected, as well as that PIP assay is evidently one of convenient ways for measurement of collagenolytic activity. The high value in apparently proliferative tissue from Dupuytren's contracture also reflects the pathogenesis of the disease in which metabolism of collagen is believed very much increased. And by this way, it supports the usefulness of PIP assay as a measurement.

The big difference found between the PIP levels of synovium and capsule from rheumatoid arthritis in favor of the former implies that collagenolytic activity is the event occurring mostly in the proliferated synovium by disease process. It is further supported by the results of osteoarthritis which are rather similar in two kinds of tissue. And the difference among three drug groups seem to show superiority of prednisolone as an anti-inflammatory agent and/or as an suppressive agent of collagenolysis.

Since we do not have a proper group for control from quite normal human joint, it's not possible to say for sure whether the enzymatic activity is increased as a whole or not in osteoarthritis. But rather wide range of data, some of which are approaching that of rheumatoid arthritis explains the variability of degree of inflammation actually present in the joint regardless of the nature of the disease.

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