

Extracellular matrix components and soluble VCAM-1 in synovial fluid with osteoarthritis of the knee

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Abstract

It is generally accepted that the number of patients with osteoarthritis (OA) of the knee must increase in the future because of the increase of the aged. The pathogenic mechanisms of OA haven't been elucidated yet, but it is supposed that mediate pannus may cause the factors for irreversible cartilage damage in the inflammatory process. Therefore it is very important to find and predict OA at the early stage. Now there were many indices to determine joint damage with OA, for example, cytokines including IL, TNF, TGF, matrix metalloproteinase (MMPs), tissue inhibitor of metalloproteinase (TIMPs), proteoglycan (PG), hyaluronate (HA), super oxide, adhesion molecules including, ICAM-1, VCAM-1 and PECAM.

In this study, we measured that the soluble VCAM-1 (sVCAM-1), soluble collagen (sCOLL), heparansulfate proteoglycan (HSPG), uronic acid and CRP in synovial fluids (SF) with OA. A positive correlation between the level of sVCAM-1 and sCOLL ($\rho=0.652$) was noted. However there was no factor showing a correlation with CRP. It was suggested that the levels of sVCAM-1 and sCOLL in the synovial fluid were active inflammation indices of knee damage, as the different aspects from CRP. The level of HSPG also showed a unique trend because HSPG on the cell surface was quickly digested endocytosis, so that it was not suitable for the inflammatory index in synovial fluid with OA.

Key words: Osteoarthritis, Extracellular matrix, Soluble VCAM-1, Soluble Collagen, Soluble HSPG

Introduction

The pathogenic mechanisms of osteoarthritis (OA) haven't been elucidated yet, but mediate pannus is reasonable to assume that the inflammatory character in the adjacent synovial cell exudate during the inflammatory process may constitute the factors causing irreversible damage of cartilage with osteoarthritis (OA)^{1,2)}. That is the reason why it is very important to find and predict OA at the early stage. At present, there were many indices of cell-matrix

interaction, mediated by integrins^{3,4)}, and the elevations of their levels are significant in the inflammatory response by regulating adhesion of leukocytes to vascular endothelial cells⁵⁾.

Vascular cell adhesion molecule-1 (VCAM-1)^{6,7)} is expressed in the cells such as endothelium, dendritic cells, macrophages. The expression of VCAM-1 and the other adhesion molecules (ICAM-1, E-selection) was controlled by inflammatory cytokines and the other mechanisms. Recent in vitro and animal studies have

suggested the expression of VCAM-1, ICAM-1 and E-selection on the endothelial cell surface upon stimulation with different combinations of interleukin-1 β (IL-1), tumor necrosis factor α (TNF) metalloproteinase and proteases in the synovium tissue and vessel⁸⁻¹¹. And it is useful to determine of hyaluronate¹² that the increasing of joints destruction of the knee with OA.

In this study we reported that sVCAM-1, soluble collagen, (sCOLL), soluble heparan sulfate proteoglycan (HSPG) and hyauronate (uronic acids) were extracellular matrix components in synovium with OA.^{13,14}. We demonstrated that the level of sVCAM-1, sCOLL and HSPG in synovial fluid represented knee damage with OA. And compaire with the concentration of CRP which is an acute inflammatory index in the synovial fluid resulting of knee damage with OA.

Materials and Methods

1). Human synovial fluid

Human synovial fluid was obtained from patients with OA : 7 samples from male at the age between 45 and 79 ; 9 samples from female at the age between 38 and 82. Collected samples were centrifuged at 3,000 rpm to remove the cells and particulate matter. Aliquots of the supernatant were frozen and stored at -80°C .

2). Assay of soluble vascular cell adhesion molecule-1 (sVCAM-1)

We measured the sVCAM-1 in SF with OA by indirect enzyme immunosorbent assay (British Bio-technology Ltd., U.S.A.) and the concentration of sVCAM-1 ($\mu\text{g/ml}$) was measured the OD at 405nm.

3). Assay of soluble collagen (sCOLL)

Sircol Collagen Assay (Biocolor Ltd., N Ireland) was used to measure sCOLL (mg/ml) in SF with OA, determining the red color at 540nm derived from binding of collagen to sirius red

dyes.

4). Measurement of soluble heparansulfate proteoglycan (HSPG)

HSPG in SF with OA was measured using Lipoprotein lipase-ELISA method (OKAMOTO et al., in press)¹⁵ at 405nm.

5). Measurement of Uronic acids (URON)

Uronic acids (Hyaluronate) in SF ($\mu\text{g/ml}$) was determined using Bitter and Muir's procedures at 530nm¹⁶.

6). Measurement of CRP

CRP ($\mu\text{g/ml}$) in SF was detected with nephelometric assay (Hoexist Japan).

7). Measurement of total protein in SF (TP)

Total protein in SF was measured using refracting scope (g/dl).

8). Statistical methods

Nonparametric methods were employed. Mann-Whitney U rank sum test (p value) and Spearman's correlation tests (ρ value) for all data were performed by Stat View J-4.5.

Results and Discussion

The level of indices mentioned above are ; sVCAM-1 : 1.007 ± 0.118 ($\mu\text{g/ml}$), sCOLL :

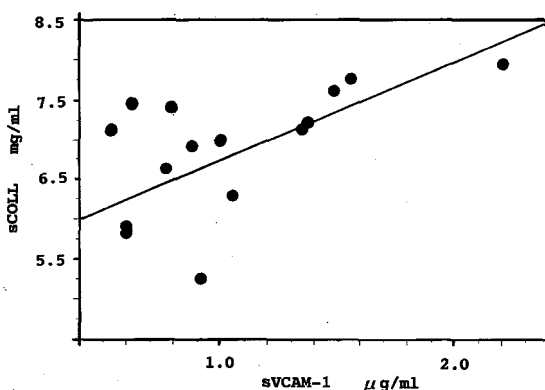


Fig. 1 Positive correlation between the concentrations of soluble vascular cell adhesion molecule-1 (sVCAM-1) and soluble collagen (sCOLL) in synovial fluid with patients of osteoarthritis ($\rho=0.652$, $p=0.01$).

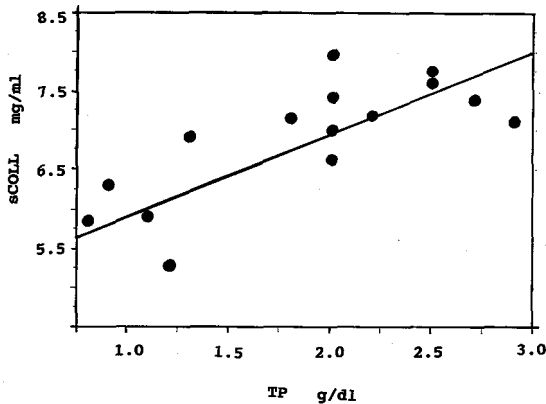


Fig. 2 Positive correlation between the concentrations of soluble collagen (sCOLL) and total protein (TP) in synovial fluid with patients of osteoarthritis ($\rho=0.737$, $p<0.05$).

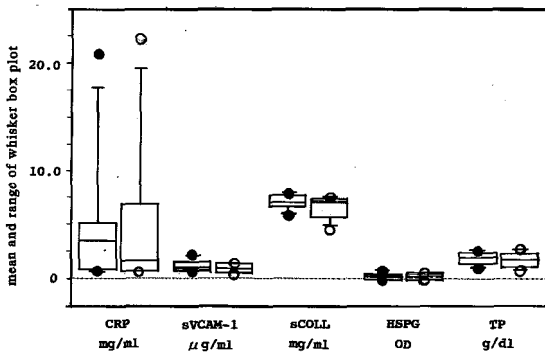


Fig. 3 There were no remarkable correlation between the data from male (●) and female (○) for soluble VCAM-I (sVCAM-1), soluble collagens (sCOLL), heparansulfate proteoglycans (HSPG), total protein (TP) and C reactive proteins (CRP) in synovial fluid with patients of osteoarthritis.

6.759 ± 0.235 (mg/ml), HSPG : 0.232 ± 0.040 (OD), URON : 159.0 ± 17.251 (pg/ml). and CRP : 5.333 ± 1.818 (µg/ml).

There were positive correlation between the levels of sVCAM-1 and sCOLL (Fig.1, $\rho=0.652$ $p=0.01$) in SF with OA, and between the level of sCOLL and total protein (Fig.2, $\rho=0.737$, $p<0.05$).

There was no sex difference among sVCAM-1, sCOLL, HSPG, URON, total protein and CRP in SF with OA (Fig.3). And then, there

was no correlation between CRP and other parameters.

Several studies have demonstrated the elevation of the level of adhesion molecules, interleukins, extracellular matrix components and MMPs in acute or chronic inflammatory diseases including osteoarthritis of the knee.

In this study, we discussed the pathogenic mechanisms which mediate pannus was reasonable to assume that the inflammatory character in the adjacent synovial cell exudate during the inflammatory process might constitute the factors causing irreversible cartilage damage of osteoarthritis (OA).

Now there were many indices to detect the cell-matrix interaction mediated by integrins. Their levels show the increase during the inflammatory response because of regulating adhesion of leukocytes to vascular endothelial cells which activate metabolism for restoration and reproduction in the synovium.

Vascular cell adhesion molecule 1 (VCAM-1) is expressed in endothelium of the knee. The expression of VCAM-1 and the other adhesion molecules (ICAM-1, E-selectin, PECAM etc) was controlled by inflammatory cytokines and other mechanisms. It suggested the expression of adhesion molecules on endothelial cell surface upon stimulation with different combination of IL-1, TNF. MMPs and proteinases in the synovium tissue and vessels, so that many molecules of extracellular matrix components might be released in SF.

In general, the level of proteoglycans in joint fluid decrease with the progress of cartilage destruction with osteoarthritis and rheumatoid arthritis, partly as a result that the amounts of remaining cartilage in the joint was reduced.

In this study we measured sVCAM-1 as an adhesion molecule. Soluble collagen (sCOLL), soluble heparan sulfate proteoglycan (HSPG)

and uronic acid (URON) were extracellular matrix components and released in joint fluid accompanied with the inflammation of the knee.

There was positive correlation between sVCAM-1 and sCOLL, while there was weak negative correlation between sCOLL and HSPG. It was suggested that the levels of sVCAM-1 and sCOLL in SF might indicate similar trend toward destruction and reproduction when the joint tissue was damaged. But the level of HSPG had a different aspect that the HSPG on the cell surface was quickly digested by endocytosis (M. YANAGISHITA 1992)¹⁷⁾, so that the detailed tendency of HSPG in SF remained being unknown.

The elevation of sVCAM-1 and sCOLL in SF that may be an inflammatory indices to predict OA. Recent studies of the acute phase response in OA suggest that the concentration of CRP are elevated in OA¹⁸⁾. However there was no correlation between the concentration of CRP and other subjects in this study. It may be an acute-phase inflammation index that the concentration of CRP in the SF with OA. Therefore it is necessary for determining the disease activity to accumulate data including the other parameter obtained from the patients with osteoarthritis.

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変形性膝関節炎患者膝関節液中の細胞外マトリックス成分と可溶性 VCAM-1

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要 約

変形性膝関節炎 (OA) 患者は、高齢化社会の到来とともに今後ますます増加するものと考えられる。OA の発症メカニズムについては、関節軟骨の破壊・修復やパンプス形成に関わる多くの関連因子 (サイトカイン, マトリックス分解酵素, 分解酵素抑制因子, プロテオグリカン, 活性酸素, 細胞接着因子など) について、多面的に研究が進んでいる。しかし、膝関節の炎症初期の病態のメカニズムについては、なお詳細に解明されておらず、関節炎初期の炎症指標の検索が今後さらに必要と考えられる。

本研究で私たちは、OA 患者膝関節液中の細胞外マトリックス構成成分であるコラーゲン、ヘパラン硫酸プロテオグリカン、ウロン酸および炎症の最も初期に血管内皮細胞に出現する VCAM-1 等の可溶性成分濃度を測定した。患者膝関節液 (SF) 中の可溶性コラーゲン量と可溶性 VCAM-1 (sVCAM-1) 量とは有意な正の相関関係が ($\rho=0.652$) 観察された。しかし、ヘパラン硫酸プロテオグリカン (HSPG) と可溶性コラーゲン (sCOLL) 値間には有意な相関関係は確認されなかった。また、一般に炎症の指標として用いられる CRP 値とも比較検討したが、CRP 値と他の測定項目値とは一定の相関関係は存在しなかった。

以上の結果より、OA 患者の SF 中の炎症マーカーとして、急性炎症時には従来の炎症指標蛋白質である CRP 値が有力であるという Sipe JD の報告から推測すると sCOLL と sVCAM-1 は CRP 値と一定の相関を示さないことより、CRP の動態とは異なった炎症指標としての有用性が考えられる。しかし、HSPG はエンドサイトーシスにより速やかに代謝されるため SF 中への解離が少なく、炎症指標としての可能性は低いと考えられる。

キーワード: 変形性膝関節炎, 細胞外マトリックス, 可溶性 VCAM-1, 可溶性コラーゲン, 可溶性ヘパラン硫酸プロテオグリカン
