EVALUATION OF SOME ANGIOGENIC STIMULATORS IN RHEUMATOID AND OSTEOARTHRITIS PATIENTS

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ABSTRACT

Hypothesis: Angiogenesis, the development of new blood vessels, is important in rheumatological disorders. Vascular endothelial growth factors (VEGF), interleukin-8 (IL-8) and nitric oxide (NO) are important angiogenic stimulators that play a crucial role in angiogenesis.

Methodology: Serum levels of VEGF, IL-8 and NO were determined in 20 rheumatoid arthritis (RA) patients; 32 osteoarthritis (OA) patients and 10 completely healthy individuals comparable to patients in age and serving as a control group. The serum levels of these bioindices were correlated to criteria of activity of RA and OA.

Results: The study revealed significantly increased levels of angiogenic factors in RA and OA as compared to controls. However, the levels of VEGF and IL-8 were insignificantly higher in RA as compared to OA. But NO levels were significantly higher in RA as compared to OA. The levels of these indices were related to activity criteria of RA, being higher in patients with greater number of joints affected, advanced function loss or x-ray grade, and correlated significantly with ESR and articular index as well.

In OA, VEGF, IL-8 and NO reflected the activity of the disease, being higher in advanced grades of disease and correlated with ESR. The sources of these angiogenic stimulators are multiple. They can be derived from a variety of activated cells (including chondrocytes, synovial cells and macrophages).
**Conclusion:** The effect of some currently used antirheumatic agents could be attributed to their effects on those angiogenic stimulators. New pharmacologic interventions using new antiangiogenic drugs could be helpful in rheumatology.

**INTRODUCTION**

Rheumatoid arthritis (RA) is a systemic disease whose features include extensive inflammation in regional synovium. The characteristic pathological findings of RA synovitis are aberrant proliferation of synovial lining cells, neovascularization by small vessels, accumulation of inflammatory cells in the synovium, and subsequent degradation of cartilage matrix Muro et al. (1999).

Angiogenesis, the development of new blood vessels is important in some pathological states. It may also contribute to the pathogenesis of some conditions. In RA, the hyperplastic synovial pannus behaves like a solid tumor, because it is rich in blood vessels invades the joint, and destroys cartilage and bone (Colville-Nash & Scott, 1992).

Angiogenesis is a complex process involving remodeling of the extracellular matrix and endothelial cell proliferation that results in the formation of new blood vessels. Active angiogenesis occurs during embryonic development as well as during wound healing and neoplasia (Folkman & Shing, 1992). Such angiogenesis is known to be regulated by a number of growth factors, including basic fibroblast growth factor, vascular endothelial cell growth factor (VEGF), platelet derived growth factor and transforming growth factor Yoneda et al. (1998).

Among others, VEGF, a 43-46 kda heparin binding dimeric glycoprotein, is a potent endothelial cell mitogen and motogen and as a mediator of increased vascular permeability by binding to its receptors Brown et al. (1997) a recent study also found that VEGF was also able to rescue senescent endothelial cells, restoring them to proliferation, and was able to delay significantly senescence in endothelial cells (Watanabe et al., 1997).

Recently, several investigations indicated the involvements of the VEGF in the pathogenesis of synovitis in RA. It is demonstrated that concentration of VEGF in synovial fluid was high and that VEGF positive cells existed in synovial tissues in RA patients (Fava et al., 1994 and Koch et al., 1994). Furthermore, Nagashima et al. (1995) demonstrated VEGF expression on synovial tissues from RA patients reverse transcription
polymerase chain reaction (RT-PCR) and in situ hybridization. These results indicated that VEGF might be involved in synovitis of RA. In a recent study, Harada et al. (1998) indicated that the serum concentration of VEGF was significantly higher in RA patients than in controls.

The serum concentrations correlated with disease activity. In a similar manner, Kikuchi et al. (1998) reported increased serum VEGF levels in RA patients.

Neutrophil attractant protein-1 interleukin-8 (IL-8) is a 72-aminoacid peptide which has been shown to degranulate neutrophils and to show respiratory burst in vitro (Pevri et al., 1986) and to be chemotactic for neutrophils and lymphocytes in vitro and in vivo (Larsen et al., 1989). Interleukin-8 is also angiogenic and induces proliferation and chemotaxis of endothelial cells (Koch et al. 1992). According to De-Benedetti et al. (1999), elevated serum IL-8 levels were found in RA patients. Also, Feldmann et al. (1996) and Charles et al. (1999) reported that IL-8 is present in significant quantities in circulation of patients with R.A. The source(s) of IL-8 in the sera of these patients are multifactorial.

Nitric oxide (NO) is a well-known regulator of homeostatic processes and host defenses (Dawson & Dawson, 1995) in addition to its well-documented vasoactive effect (Ignaro et al. 1987) and neurotransmitter properties (Dawson & Dawson, 1995). NO produced by macrophages and endothelial cells plays a major role in host defense against bacteria and tumor cells (Hibbs et al. 1987). It may interact with oxygen-derived radicals to generate molecules that can nitrosate proteins; modify their function, and produce DNA damage (Crow & Beckman, 1955). Recent evidence suggests that NO is generated in the inflamed joint via Larginine-ON pathway in RA patients (Farrell et al. 1992). According to these investigators serum nitrite (the breakdown products of NO) concentrations were elevated in RA patients as compared with controls. Also, Grabowski et al. (1996) and Hilliguin et al. (1997) reported increased nitrite levels in sera of RA patients as compared with controls. Although NO production can be induced in some cells by the actions of proinflammatory cytokines and by bacterial endotoxin and lipopolysaccharide (O’Donnell & Liew, 1994). The role of NO in inflammatory joint disease is still uncertain.

Osteoarthritis is a common degenerative joint disease affecting the articular chondrocytes. The major feature is erosion of the cartilage, which may produce breakdown of the underlying subchondral bone (Kraus, 1997). The role of angiogenesis in this disorder still needs to be clarified.
According to Harada et al. (1998) the levels of the angiogenic cytokine, VEGF were insignificantly different from controls. However, Kikuchi et al. (1998) reported significantly elevated VEGF serum levels in OA patients in comparison with controls. Pulsatelli et al. (1999) reported that IL-8 production by chondrocytes of OA patients was similar to chondrocytes of RA patients. However, Furuzawa-Carballeda & Alcocer-Varela (1999) showed that IL-8 expression levels are higher in synovial tissues from RA patients that in similar tissue from OA patients. High concentrations of NO have been demonstrated in the synovial fluid of OA patients (Farrell et al. 1992) and OA Cartilage was shown to produce NO spontaneously (Amin et al., 1995).

Aim of Work:

The current study was conducted to substantiate the role of angiogenic stimulators VEGF, IL-8 and NO in RA patients in comparison with OA and Healthy controls. The levels of these indices would be correlated with clinical criteria and disease activity.

PATIENTS AND METHODS

Serum samples were obtained from 20 active RA patients (19 females and one male, female: male 95: 5, age range 20-65 years, mean 41.6 years), that fulfilled the American College of Rheumatology Criteria (Arnett et al., 1988) and who were not taking oral corticosteroids. The study also included 32 primary OA patients (20 females and 12 males, female: male 62.5: 37.5, age range 38-70 years, mean 52.4 years). The diagnosis of OA was based on clinical and radiological features.

Diagnostic accuracy for RA and OA patients was assured by clinical review and concurrent laboratory assessment. Clinical assessment included thorough clinical examination with special emphasis on joints examination to determine disease activity score which equals: 0.53938 x (square root of Riptide score) + 0.06465x (number of swollen joints) + 0.33 x LN (ESR) + 0.224 (Van der Heijde et al., 1990). Routine clinical and laboratory investigations in the form of chest x-ray, echo-cardiography, abdominal sonography, ECG, complete urine analysis, ESR, full blood counts, C-reactive protein (CRE) level and rheumatoid factor (RF) titre, were also performed.

From each patient as well as from the 10 completely healthy persons comparable in age (14.0-60.0 years) to patients 10-cc blood were withdrawn
by venipuncture, sera were separated after centrifugation and kept in aliquots at –70°C till further analysis.

VEGF serum levels were determined with sandwich enzyme immunoassay (ECA) using CYELISATM human VEGF kits, which measure the free forms of the cytokine. The kit is supplied by CYIMMUNE science Inc., USA cat. N 5.587.294. IL-8 levels were determined with enzyme linked immunosorbent assay (ELSSA) using kits supplied by Immunotech Company France Cat. N. 2237. Nitric oxide was determined with evaluation of its oxidation products nitrates and nitrites where nitrates were reduced to nitrite with cadmium fillings; the total concentration of nitrate was then measured by using Griess reaction (Davidson & Woolf, 1978 and Thomsen et al., 1992).

Statistics:

Data obtained were statistically analyzed using student t-test for normally distributed data and Mann-Whitney test for skewed date. Spearman rank correlation was also used for statistical analyses. A p< 0.05 was considered significant.

RESULTS

Table (1): VEGF IL-8 and NO level in RA patients in comparison with OA patients and healthy controls.

<table>
<thead>
<tr>
<th>Angiogenic factors</th>
<th>Controls (n=10)</th>
<th>RA (n=20)</th>
<th>OA (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF Pg/ml</td>
<td>Mean # S.E. Median range</td>
<td>Mean # S.E. Median range</td>
<td>Mean # S.E. Median range</td>
</tr>
<tr>
<td>334.55#94.27 55.5-980.8 260.0</td>
<td>2294.7#320.84 701.3-4976.9 p&lt;0.001 N.S</td>
<td>1765.#202.82 951.9&lt;4116.2 p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>IL-8 Pg/ml</td>
<td>Mean # S.E. Median range</td>
<td>Mean # S.E. Median range</td>
<td>Mean # S.E. Median range</td>
</tr>
<tr>
<td>62.22#9.69 25.7-110.5</td>
<td>353.88#39.02 156.7-711-10 p&lt;0.001 N.S</td>
<td>327.47#26.27 99.7-720.8 p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>NO Mmol/L</td>
<td>Mean # S.E. Median Range</td>
<td>Mean # S.E. Median Range</td>
<td>Mean # S.E. Median Range</td>
</tr>
<tr>
<td>7.15 #0.66 6.85 3.3-10.1</td>
<td>30.97 # 1.99 18.5-49.1 p&lt;0.001 p&lt;0.05</td>
<td>25.24 # 0.99 12.9-33.9 p&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

N.B.: Statistical analysis was performed by student test. N.S=>0.05 S=<0.05

As evident in table (1), the serum levels of VEGF IL 8 and NO were significantly increased in patients with either OA or RA in comparison with
controls. RA patients showed higher levels of VEGF IL–8 and NO compared with OA, but the differences reached the level of significance in case of NO only. The differences between the 3 groups are further clarified from individual values shown in Fig (1) to (3). RA patients having fever, more than 4 affected joints, advanced functional or x-ray grade showed higher levels of all angiogenic stimulators, the difference reached the level of significance in case of VEGF in patients with grade III functional all class (table 2). Meanwhile patients with x-ray grade III & IV osteoarthritis also showed higher levels of VEGF, IL 8 and NO compared with patients with x-ray grader I & II (table 3).

Correlations between various indices are shown in Fig (6) to (12). In both RA and OA patients, significant positive correlation existed between VEGF and either IL-8 or NO (Figs. 6 & 7) Also, IL-8 correlation significantly in a positive manner with NO in both patients groups (Fig. 8). Significant positive correlation existed between ESRI and VEGF in either RA or OA (Fig. 9). In OA patients ESRI and ESR2 correlated significantly with VEGF, IL-8 and NO (Fig 10, 11 and 12). In RA patients, significant positive correlations existed between VEGF, IL-8 and NO and articular index.

![Fig. (1): Individuals of VEGF levels in the studied groups.](image1)

![Fig. (2): Individuals of IL-8 levels in the studied groups.](image2)
Fig. (3): individuals NO levels in the studied groups.

Fig. (4): individuals of ESR1 levels in the studied groups.

Fig. (5): individuals ESR2 levels in the studied groups.

Fig. (6) Correlation between VEGF and IL-8 in RA and OA groups.
Fig. (7): Correlation between VEGF and NO in RA and OA groups.

Fig. (8): Correlation between IL-8 and NO in RA and OA groups.

Fig. (9): Correlation between VEGF and ESR1 in RA and OA groups.

Fig. (10): Correlation between VEGF and ESR2 in OA groups.
Table (2): VEGF, IL-8 and NO levels in RA according to clinical criteria.

<table>
<thead>
<tr>
<th>Clinical criteria</th>
<th>VEGF Pg/ml</th>
<th>IL-8 Pg/ml</th>
<th>NO Mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean # S.E.</td>
<td>Median</td>
<td>n</td>
</tr>
<tr>
<td>Fever Present</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>Mean</td>
<td># S.E.</td>
<td>Median</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2054.7</td>
<td>454.22</td>
</tr>
<tr>
<td></td>
<td>339.71</td>
<td>58.92</td>
<td>297.6</td>
</tr>
<tr>
<td></td>
<td>28.1</td>
<td></td>
<td>19.3-49.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 4 (n=10)</td>
<td>Mean # S.E.</td>
<td>Median</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1986.7 #439.27</td>
<td>1524.5</td>
</tr>
<tr>
<td></td>
<td>2891.9</td>
<td>30.86</td>
<td>194.9</td>
</tr>
<tr>
<td></td>
<td>18.5-49.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;4 (n=10)</td>
<td>Mean # S.E.</td>
<td>Median</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2602.8 #</td>
<td>469.66</td>
</tr>
<tr>
<td></td>
<td>323.4</td>
<td>199.5-711.1</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Joints affected</td>
<td>Mean # S.E.</td>
<td>Median</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1723.9 #</td>
<td>305.13</td>
</tr>
<tr>
<td></td>
<td>1813.9</td>
<td>261.35</td>
<td>182.8-538.1</td>
</tr>
<tr>
<td></td>
<td>35.66 #3.87</td>
<td>36.5</td>
<td>19.3-49.1</td>
</tr>
<tr>
<td>Functional Classification</td>
<td>Mean # S.E.</td>
<td>Median</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3151.0 #551.8</td>
<td>2964.7</td>
</tr>
<tr>
<td></td>
<td>1813.9</td>
<td>297.6</td>
<td>156.7-711.10</td>
</tr>
<tr>
<td></td>
<td>34.34 #3070</td>
<td>31.9</td>
<td>18.5-49.1</td>
</tr>
<tr>
<td>III (n=8)</td>
<td>Mean # S.E.</td>
<td>Median</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2882.3 #527.47</td>
<td>2931.2</td>
</tr>
<tr>
<td></td>
<td>28.1</td>
<td></td>
<td>19.3-49.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-ray grading</td>
<td>Mean # S.E.</td>
<td>Median</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2882.3 #527.47</td>
<td>2931.2</td>
</tr>
<tr>
<td></td>
<td>28.1</td>
<td></td>
<td>19.3-49.1</td>
</tr>
</tbody>
</table>

N.S.: Statistical analysis was performed by student test except where Mann-Whitney test was used.
N.S.>=0.05 S=<0.05
Fig. (11): Correlation between IL-8 and ESR1 & ESR 2 in OA groups.

Fig. (12): Correlation between NO and ESR 1& ESR 2 in OA groups.

Table (3): VEGF, IL-8 and NO in osteoarthritis in relations to X-ray grading.

<table>
<thead>
<tr>
<th>X-ray</th>
<th>VEGF (Pg/ml)</th>
<th>IL-8 (Pg/ml)</th>
<th>NO (Mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I &amp; II (n=17)</td>
<td>Mean # S.E.</td>
<td>1264.1 #26377</td>
<td>285.09 #41.85</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>711.4</td>
<td>236.7</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>591.9-4116.2</td>
<td>99.7-720.8</td>
</tr>
<tr>
<td>III &amp; IV (n=15)</td>
<td>Mean # S.E.</td>
<td>2334.0 #124651</td>
<td>375.5 #26.12</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>2273.3</td>
<td>395.9</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>769.3-3851.1</td>
<td>208.7-586.1</td>
</tr>
<tr>
<td></td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

N.B.: Statistical analysis was performed by Mann Whitney test.
N.S=>0.05

**DISCUSSION**

Angiogenesis involves the degradation of extracellular matrix and the migration, proliferation and formation of tubes by endothelial cells *Risau, (1997).* Various factors, such as heparin binding growth factors, angiogenin, interferons, transforming growth factors, interleukins, and prostaglandins, are involved in angiogenesis in RA (*Colville-Wash and Scott, 1992*). Vascular endothelial cell growth is a secreted protein that
stimulates endothelial cell proliferation and migration, it also increases vascular permeability (Ferrara and Davis-Smyth, 1997). In some cytokine-induced neovascularization, VEGF mediates the angiogenesis Yoshida et al., (1997) and may be involved in the pathogenesis of RA (Koch et al., 1994; Nagashima et al., 1995 and Fava et al., 1997). The present study showed that the serum levels of VEGF in RA patients were significantly increased in comparison to controls. However, despite the higher levels in RA patients in comparison to the corresponding levels in OA, the differences did not reach the level of significance. Mean while, OA patients also showed significantly higher serum VEGF levels compared to controls (table 1) Similar to present findings, (Harada et al., 1998; Kikuchietal 1998 and Paleolog et al., 1998). Reported significantly higher serum levels of VEGF in RA patients compared to controls and OA patients. Meano et al., (1999) reported significantly increased serum levels of VEGF in patients with juvenile RA compared with healthy controls. In RA patients, VEGF reflected number of affected joints, functional and x ray grading and exhibited significant correlation with ESRI and articular index (Table 2 & Fig. 9). According to Harada et al., (1998), of markers for disease activity, serum VEGF correlated significantly with CRP, but serum concentrations of VEGF did not correlate with WBC, ESR, and RF titer.

In our study RF titer also did not correlate with VEGF. However, Harada et al., (1998) could not find any relationship between serum VEGF levels and larsen’s radiographic analysis Larsen et al., (1997). Paleolog et al., (1998) also reported significant correlation between VEGF serum levels and diseases activity in RA.

The sources of VEGF in sera of RA patients are multiple. VEGF is abundant in synovium and synovial fluids, where it contributes to vascular permeability. Synovial fibroblasts seems to be an important source of VEGF especially under effect of cytokines Berse et al., (1999). VEGF especially its isoform is upregulated selectively in RA synovial cell Ikeda et al., (2000). Neutrophils infiltrating RA synovial fluid are important source of VEGF Kasama et al., (2000). In an interesting study, Yamada et al., (1998) suggested that mast cells in synovial membrane are also important source of VEGF. Besides its local production, VEGF seem to be also produced by peripheral monocytes Bottomley et al., (1999).

It should be considered that the synovitis in RA resembles tumor growth, and the inflammation invades and destroys articular cartilage, subchondral bone, tendons, and ligaments Harris et al., (1993). Abundant new capillary growth is important in the development of synovitis in RA.
Harris et al., (1993). This could explain the significant correlation observed between VEGF serum levels and disease activity observed in the present study as well as in the studies conducted by (Harada et al., 1998 and Paleolog et al., 1998).

In recent years, analysis of cytokine production in RA has attracted particular interest since many cytokines are involved in the regulation of the immune and the inflammatory response Feldmann, (1996). They function as communication signals in the complex bidirectional interaction between leukocytes and endothelium a pivotal event in the development of inflammatory injury Fajardo, (1999). They also act as mediators of many vital biological processes such as immunity, cell growth, repair fibrosis and angiogenesis Feldman and Maini, (1999). There are many families of cytokines which contain over 100 known molecules and many more in the process of being identified they are produced by many (perhaps all) cells upon activation and act locally with notable exceptions. Cytokine inhibitors are concomitantly present (e.g. soluble receptors). Because they act on high affinity receptors, cytokines are very potent Feldman and Maini, (1999). Several groups of investigators began to ask which cytokines were up regulated in the joints of people with active RA (Miossec et al., 1990, Koch et al., 1991 and Fva et al., 1994) Several proinflammatory cytokines including IL-8 are expressed in abundant amounts in RA joints Feldman and Mini, (1999). In the present study, serum levels of IL-8 were found to be significantly increased in RA patients and OA compared to controls. Despite the higher levels of IL-8 in sera of RA patients compared to OA, the differences between the 2 groups did not reach the level of significance. Among criteria of disease activity, IL-8 was higher in patients with more number of joints involved, advanced functions or x ray grading (Table 2). It correlated significantly with ESRI and ESR II (Fig. 11) and articular index (r = 0.79 & p< 0.001). No corelation of IL-8 with RF titre could be observed. Similar to present findings, Feldmann and Brennan (1996); Charles et al., (1999) reported the presence of significant amount of IL-8 in RA patients, which were reduced after treatment. De-Benedetti et al., (1999) reported elevated serum levels of IL-8 in patients with juvenile RA as well as adult type.

The sources of IL-8 in sera of patients with either RA or OA are multiple. Although cells of the immune system are considered to be their primary sources hey can also be produced by other cell types such as fibroblasts endothelial or even epithelial cells thus, in the inflamed synovium, monocytes as well as type A and type B synoviocytes have been
shown to produce large amounts of cytokines particularly pro-inflammatory cytokines including IL-8 (Arend and Dayer, 1995; Koch et al., 1995 and Brennan et al., 1996). Also the cells in RA synovial tissue could produce IL-8 Steiner et al., (1999). Takahasi et al.,(1999) reported that IL-8 generated from synovial lining cells and macrophages may participate to the inflammatory process in the early synovitis of RA, as it is produced in the early phase of the disease. In the active phase, these investigators, observed prominent production in most components of the joints such as synovial lining cells, migrated monocytes, sublining fibroblastoid cells, endothelial cells or migrated neutrophils. However, the expression of IL-8 was low in fibrotic synovitis in RA. Immune complexes present in sera of RA patients interact with resident joint synovial fibroblasts (synoviocytes) and induce the expression of IL-8, which in its turn could play a key role in inflammation by recruiting leukocytes to synovial tissue and fluid-and subsequently contributing to joint disease Khalkhali-Ellis et al., (1999). In a recent study, Pulsatelli et al., (1999) reported that chondrocytes in RA patients and OA produce IL-8. Proinflammatory factors (IL1β, TNFα) effectively upregulate IL-8 production. There was no statistical difference between chondrocytes of RA and OA in amount of IL-8 production. This agrees with our criteria where no significant differences in serum IL-8 levels between RA and OA (table 1). The chondrocyte derived chemokine, IL-8 may play a role in triggering the mechanisms involved in the pathogenesis and persistence of joint diseases. This was supported by the higher levels of IL-8 with advanced disease in either RA or OA (Table 2 and 3). However, Furuzawa et al., (1999) reported that the expression levels of IL-8 are higher in synovial tissue from RA patients than in similar tissue from OA patients. According to Borzi et al., (1999), in OA and RA patients, an enhanced IL-8 expression was observed, in chondrocytes. Besides its local production within the inflammed joints, Rodenburg et al., (1999) postulated that monocyte derived macrophages of RA patients possess innate hyper-responsiveness contributing to the high IL-8 levels present in the synovial fluid of RA joints and is implicated in the chemotactic gradients leading to the homing of leucocytes to the joints. In this respect, it should be considered that the significant positive correlation observed between VEGF and IL-8 in either RA or OA in the present study (Fig. 6) suggest their common source and or mechanism of action. IL-8 belong to a new family of cytokines that appear to have proinflammatory and reparative activities Baggiolini et al., (1992). These cytokines in their monomeric forms are all less than 10 KDa and are characteristically basic heparin-binding proteins. This family displays four highly conserved cysteine amino acid residues,
with the first two cysteines separated by one nonconserved amino acid residue. In general, these cytokines appear to have specific chemotactic activity for neutrophils. Because of their chemotactic properties and the presence of CXC cysteine motif, these cytokines have been designated the CXC chemokine family. Although numerous in vivo and in vitro investigations have shown the importance of IL-8 in acute inflammation as a chemotactic/activating factor for neutrophils only recently has it become apparent that this CXC chemokine may be an important mediator of angiogenic activity.

This, IL-8 has been found by several investigators to be a potent angiogenic factor (Koch et al., 1992; Strieter et al., 1992 and Hu et al., 1993). Recombinant IL-8 mediates both endothelial cell chemotactic and proliferative activity in vitro and angiogenic activity in vivo. IL-8 induced angiogenic activity similar to VEGF Koch et al., (1992). The presence in IL-8 of the sequence Glutamine-Leucine-Arginine (the ELR motif), an amino acid sequence that appear to be important in ligand/receptor interactions on neutrophils is the putative domain that dictates the angiogenic activity (Hebert et al., 1991 and Clark-Lewis et al., 1993).

Nitric oxide is a free radical synthesized from L-arginine by NO synthase (NOS) Moncada, (1992). The endothelial isoform of NOS (eNOS) synthesizes small amounts of NO that induces peripheral vasodilatation, whilst the nearly ubiquitous inducible isoform (iNOS) produces large amounts of NO with pro-inflammatory action (Anggard, 1994). NO is thought to play a role in a number of inflammatory processes, including RA Ueki et al., (1996). In the present study, the levels of NO in the form of its breakdown products nitrites and nitrates were significantly increased in patients with either OA or RA compared to controls. However, the levels in RA were significantly higher than the corresponding levels in OA (Table 1). The levels reflect RA activity being higher in cases with more number of joints involved, advanced functional and x-ray grades as well (Table 2). It’s levels correlate with ESRI 2 significantly (Fig. 12) and also with articular index (r= 0.71 & P< 0.001). In OA patients, NO levels were higher in cases with advanced x-ray grading. It correlated significantly with ESR 1 & 2(Fig. 12). In agreement with our findings, increased concentrations of nitrites or nitrates, or both (Farrel et al., 1992; Miesel et al., 1993; Kaur & Halliwell, 1994 and Stichtenoth et al., 1995) have been detected in sera of RA patients. Moreover, increased concentrations of nitrites have been demonstrated in the joint fluids of both OA and RA patients (Farrell et al., 1992; Miesel & Zuber, 1993 and Kaur & Halliwell, 1994). Other indicators
of increased NO production in sera of RA patients or OA in the form of nitrotyrosine (Kaur and Halliwell, 1994 and Oates et al., 1999) or N^G^-hydroxy-L-arginine, a NO synthase metabolite released from NO producing cells and accumulates in the circulating blood (Hecker et al., 1995) also have been reported to be increased in sera of RA patients Wigand et al., (1997).

The sources of NO in inflammed OA or RA joints are multiple. Thus, Sakurai et al. (1995) First demonstrated inducible NOS expression in both RA and OA synovocytes and chondrocytes. Hayashi & Coworkers (1997) demonstrated greater amounts of iWOS in cells residing in superficial zones of cartilage in stimulated cartilage. McInnes et al.. (1996) have reported that synovial membrane cultures from OA and RA patients spontaneously produce NO. In a recent study, Amin et al. (1999) reported the presence of inducible NO1 in the synovial tissue and in the cartilage of patients with either OA or RA NO has several catabolic effects on chondrocytes Amin and Abramson, (1998). It inhibits the synthesis of collagen and proteoglycans Cao et al., (1997) and modulates the production of metalloproteinases (Murvell et al., 1995). According to Hashimoto et al. (1999) NO production by chondrocytes causes apoptotic cell death. This may contribute to matrix loss and calcification.

Besides its local production, NO production by blood mononuclear cells of RA patients was demonstrated by St-Clair et al., (1996). This was due to systemic activation of NO synthase expression. In the present study, significant positive correlation was observed between IL-8 and NO. This could be attributed to the action of NO as a regulatory signal in the induction of production IL-8 by endothelial cells (Villarete & Remick, 1995). Moreover, both of these bioindices mediate angiogenic activity Leibovich et al., (1994).

It could be concluded that the angiogenic stimulators, VEGP, IL-8 and NO are significantly increased in sera of patients with either RA or OA, compared to healthy controls. The levels were correlated to disease activity. These angiogenic stimulators are released by a variety of activated cells (including chondrocyte, synovial cells and macrophages) represent a potential new target for pharmacologic intervention. Thus, according to Hagedorn & Bikflavi (2000), anti-angiogenic drugs will improve future therapies of OA and RA. Moreover, it should be noted that agents currently used in the treatment of rheumatic disease might affect NO activity. Thus, while auranofin reduced the response to NO, glucocorticoids and cyclosporins inhibit the induction of inducible NOS in several tissues.

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880


Angiogenic Stimulators in RA and OA


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ประเมي بعض محفزات التكوينات الوعائية الجديدة في مرض الرثيان
الفصيلي والفصالي العظمي

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يلعب التكوين الوعائي الجديد دورا هاما في أمراض الروماتيزم المختلف ومن العوامل الهامة التي تحفز التكوينات الوعائية معامل نمو الأوعية الطلائي والإنترلوكين-8 وأكسيد النيترويز وتم تقدير مستويات هذه العوامل في أمصال 20 مريضا بالرثيان الفصيلي وكذلك 32 مريضا بالفصالي العظمي و10 أشخاص أصحاء كمجموعة ضابطة متماثلة في السن مع المرضى وثبتيت الدراسة وجود زيادة جوية في مستويات هذه العوامل في مرضى الرثيان الفصيلي وكذلك الفصالي العظمى بالمقارنة بالمجموعة الضابطة وكانت مستويات هذه العوامل أعلى منها في الرثيان الفصيلي عن الفصالي العظمى ولكن كانت الفروق جوية فقط في حالة أكسيد النيترويز وليست وجود علاقة بين هذه القياسات وبين نشاط المرض حيث وجد أن مستويات هذه الدراسات في حالة الرثيان المفصلي أعلى في الحالات التي يوجد بها التهاب في عدد كبير من المفاصل وتنقسم درجة المرض كما يضح من القياس الوظيفي أو حسب الأشعة ووجدت علاقة جوية بين هذه القياسات وسرعة الفصل العظمي والفصالي العظمي وفي حالة مرض الفصل العظمي في هذه الحالات ينخفض درجة الرثيان ووجود علاقة جوية بينها وبين سرعة الترسيب وكذلك مصدار متعدد لهذه العوامل مثل خلايا الفجروف وخلايا السائل الفصيلي والخلايا المлетمة وبعد الاستجابة لبعض الأدوية المضادة للروماتيزم المستخدمة حاليا ناتجة من تأثيرها على هذه العوامل المحفزة للتكنولوجيات الوعائية الجديدة هناك أدوية جديدة يمكن أن يلعب دورا في علاج الأمراض الروماتيزمية نتيجة لفصلها المضاد للتكنولوجيات الوعائية الجديدة.