Antti-inflammatory Treatments and Serum Selectin Levels in Osteoarthritis

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骨関節炎における抗炎症療法と血清selectin値

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抄録
骨関節炎（OA）は滑液性炎症を伴うリウマチ性疾患の一つである。この症状では白血球が動員され、炎症を起こした関節部に移動するが、これは、E-、P-と L-selectinなどの接着分子によって伝達される。

我々は、OA患者に対して、鉱泥浴（Abano Fangoを使用したMud Pack Treatment,MPT）と抗炎症剤での治療を行った2群で、血清 selectin濃度を指標として両療法の効果を比較した。

[対象と方法] 50人のOA患者を無作為に、MPTを行った30症例（A群）と、抗炎症剤として50mgのdiclofenacを1日に2回、毎日投与した20症例（B群）の2群に分けた。

血液試料はそれぞれの治療前後に採血し、血清E-, P-とL-selectin濃度をELISA法で測定した。

[結果] B群では、血清E-selectinは薬剤治療後に明らかに増加した。A群では鉱泥浴治療後に、血清L-selectinが有意の変化を示したが、血清P-seletinは有意の変化がなかった。

[考察] 本研究では、MPTと抗炎症剤diclofenacはOA患者においては異なった接着分子に影響することが分かった。これら両者の治療法を組み合わせることにより、リウマチ性疾患の抗炎症療法にとって、より安全で、かつ効果的であることが示唆された。

Key words: Osteoarthritis, selectins, inflammation, joints, anti-inflammatory therapies
INTRODUCTION

Osteoarthritis (OA) is a disabling chronic inflammatory disease leading to joint destruction and a common cause of significant disability, dependence and pain, mostly in the elderly.

OA consists of a spectrum of clinical entities, ranging from local chondral defects to a general condition resulting from biologic and biomechanical hyaline cartilage failure.

Many proinflammatory cytokines play a major role in cartilage destruction in this disease, and IL-1 and TNFα have been suggested as important promoters of tissue damage. They in fact contribute to accelerating the articular tissue damage and amplifying the inflammatory response.

The pathophysiologic phenomena concurring to joint damage are synovial inflammation and cartilage destruction. In this context recruited leucocytes are capable of producing a variety of inflammatory mediators while the chronically inflamed joint tissues express a pattern of cytokines and adhesion molecules that maintain the infiltration and the activation of the inflammatory cells and the tissue injury. Neutrophils migration from blood through vascular endothelium into inflamed arthritic joints is mediated by adhesion molecules.

It is known that the selectin family of adhesion molecules is particularly involved in leucocytes recruitment to sites of inflammation and tissue damage. The family consists of three cells surface molecules expressed by leucocytes (L-selectin), platelets (P-selectin) and vascular endothelium (E- and P-selectin).

The interaction between leucocytes and the endothelial adhesion molecules is a key factor in the generation of effective inflammatory and immune responses against injury. A number of adhesion molecules have been identified in synovial tissues of OA patients. Some of them are up regulated and may play an important role in the inflammatory processes of the diseased joints. In addition synovial fluids contain soluble form of many adhesion molecules such as E-selectin and L-selectin.

An important role of selectins for leucocytes rolling on the blood vessels wall and for their recirculation and entry into inflamed tissue has been established.

Anti-selectins (mAb) significantly reduce the accumulation of monocytes and neutrophils in the inflamed joints and the therapeutic use of synthetic oligosaccharides that block the structural terminal sugars on selectin molecules is currently under investigation in various inflammatory diseases.

The American College of Rheumatology (ACR) guidelines for the medical management of OA recommend the use of analgesics, COX-2 inhibitors (CSI), non steroidal anti-inflammatory drugs (NSAID) in combination with physical therapy, exercise, education and social support. Physical therapy includes the use of heat by different methods (hot tub baths, thermal bath, etc.).

Moreover, several Public Health Departments of European countries include a natural treatment such as Mud Pack Therapy (MPT) among the useful treatments of OA.

In the present study we investigated the serum levels of L-, P- and E-selectin (sL-, P- and E-selectin) in OA patients undergoing a traditional pharmacologic therapy and a cycle of
MPT to test whether a natural treatment, similarly to drugs, ameliorates inflammatory diseases and affects serum selectin values.

II MATERIALS AND METHODS

Fifty subjects were selected from a cohort of 300 consecutive outpatients consulting their general physician (GP).

After having provided their written informed consent, the patients had a physical examination and the OA diagnosis was formulated in accordance with the ACR (American College of Rheumatology) criteria for hip and knee OA.

The selected patients were randomly divided into two treatment groups consisting of a group A (30 patients aged 58.1±6.9 receiving for 12 days with a daily MPT in a thermal resort) and a group B (20 patients aged 58.9±6.6 receiving five days of nonsteroidal anti-inflammatory therapy, i.e. 50 mg diclofenac p.o. twice daily).

Group A patients were asked to discontinue any NSAID treatment and reevaluated for study inclusion after a 15 days wash-out. Successively they underwent a cycle of 12 MPT with “mature” thermal mud in a thermal establishment of Abano-Montegrotto (Padua-Italy), according to the standard protocol employed in the Euganean Basin, i.e. 15-20 minutes mud pack at 39-40°C followed by a thermal bath at 37-38°C for 10-12 minutes.

MPT is a natural anti-inflammatory treatment, consisting of a full body pack with natural clay mixed with bromine-iodine natural mineral thermal water after a process of “maturation” at 70°C for at least 2 months. The maturation is due to the development of a typical thermophilic microflora, mainly represented by blue-green algae (Cyanophyceae) and Diatoms.

These microorganisms produce a sulfolipid compound with anti-inflammatory properties which is also released in the thermal mud.

Blood samples were collected from both groups before (t 0) and after the end of the treatments (t 1) for the assay of P-, L-, and E-selectin.

The collected blood samples were promptly centrifuged and the serum was frozen at -70°C until the assay. All the samples were assayed in the same run, following the test producers’ instructions.

P-selectin was assayed by a 1.25 hour solid phase ELISA containing recombinant human sP-selectin and antibodies raised against the recombinant factor (R&D Systems, Inc., Minneapolis, MN, USA). The sensitivity of the assay was lower than 0.5 ng/ml with intra- and inter-assay coefficient of variation (CV) of 4.9 and 7.9 respectively. No cross-reactivity or interference was found with recombinant human sL-selectin or recombinant sE-selectin.

L-selectin assay employed the quantitative sandwich enzyme immunoassay technique (R&S Systems Inc., Minneapolis, MN, USA), with a sensitivity lower than 0.3 ng/ml and intra- and inter-assay CV of 3.8 and 7.9 respectively.

No cross-reactivity was found with recombinant human sP- and sE-selectins.

E-selectin assay was a 2 hours solid phase ELISA (R&D Systems Inc., Minneapolis, MN, USA) with a sensitivity lower than 0.1 ng/ml and intra- and inter-assay CV of 4.8 and 7.3 respectively. No cross-reactivity was found with natural human P-selectin.
The statistical significance of the observed differences of calculated means and standard deviations were evaluated by paired Student’s t test. All the results were included in the statistical analysis, in accord with the principles of the Intention To Treat statistical analysis (I.T.T).

Statistical methods were predefined to assess differences between the groups. The variations within the groups and correlation were also calculated.

III RESULTS

Of the total 300 screened patients 50 met eligibility criteria and were randomised in Group A (30 patients on MPT) and Group B (20 patients on diclofenac). All the patients completed the study, and the obtained results were included in the statistical analysis (I.T.T). E-selectin serum levels showed no significant changes in group A (MPT); they increased significantly in Group B (diclofenac) from $136.50\pm40.72$ to $200.50\pm42.75$ (46.89%; $P<0.01$; Fig. 1).

L-selectin values showed a significant increase: from $1203.9\pm440$ ng/ml before to $1500\pm738.51$ in Group A (24.59%; $P<0.01$, Fig. 2). In Group B L-selectin showed no changes.

The correlation analysis did not show any statistically significant result in both the groups before and after the treatments.

MPT therefore influenced sL-selectin serum values ($p<0.01$) while the pharmacologic therapy mostly influenced sE-selectin.

In particular the sL-selectin increment was 24.59% and sE-selectin is 46.89% in group B.

IV DISCUSSION

Osteoarthritis is a common clinical condition affecting up to 52% of adults over the age of 65, and pain is its cardinal feature of OA, while on the advancing of the disease there is a loss of function and increasing pain even at times of joint rest.

Although there is no known cure for OA, treatments designed for the individual patients can reduce pain, maintain and/or improve joint mobility and limit functional impairment.

The American College of Rheumatology (ACR) published recommendations for the medical management of OA of the hip and knee including non pharmacologic practices (education, exercise, physical therapy, etc.) as well as pharmacological agents, such as acetaminophen or non-steroidal anti-inflammatory drugs (NSAIDs).

The physical therapy practices are helpful for many aspects of OA management, and utilizes any method that provides heat (superficial and deep, bath, whirlpool, etc.). Such methods for treating OA patients are one of the oldest traditional forms of therapy and the results reported in many trials cannot be ignored.

The aim of balneotherapy is to soothe the pain and as a consequence to relieve patient’s suffering and make them well.

In some papers it has been demonstrated that Mud Pack Therapy (MPT) exerts an inhibitory effect on serum levels of PGE$_2$ and LTB$_4$, on IL1 and TNF$\alpha$ in OA patients and its influence
Fig. 1  Serum levels of E-selectin before and after the pharmacologic therapy. Data are shown as mean ± SD

Fig. 2  Serum levels of L-selectin before and after the pack treatment
on the pathophysiology of the inflammatory reaction and pain were mechanisms hypothesized\textsuperscript{29,30}. Until now it is not completely explained if this depends only on the heat of the treatment (\(40 \pm 1^\circ\text{C}\)) or on the combination of effects including the action of anti-inflammatory principles present in the “matured” thermal mud, produced by the typical microflora\textsuperscript{19,20}.

The goal of the present management of the patient with OA always includes a control of pain and an improvement in function and health related quality of life, by avoiding, if possible, toxic effects of the pharmacological therapy\textsuperscript{31}.

The ACR Subcommittee guidelines emphasize that these recommendations are not fixed, not rigid mandates and recognizes that the final decision about the therapeutic regimen for an individual patient is determined by the treating physician\textsuperscript{17}.

Rheumatic diseases have now became a major public health and economic problem and their therapeutical strategies need to be optimised in order to define effective procedure for controlling disease symptoms, such as pain, and for stopping or slowing the disease progression\textsuperscript{31}.

In this field the cellular adhesion molecule of endothelium and leucocytes may constitute excellent markers for assessing the effectiveness of anti-inflammatory medicines\textsuperscript{32}.

Some NSAIDs are able to inhibit the adhesion of polymorphonuclear neutrophils to endothelial cells\textsuperscript{33}, which is the first step for PMN migration to the inflammatory lesions\textsuperscript{34}. This process is mediated by the selectins, a family of three adhesion molecules which concerning the leucocytes and the vascular system.

Selectins play an important role for the rolling of leucocytes on the blood vessel walls and for lymphocyte recirculation as well as leucocyte entry into inflamed tissue\textsuperscript{35}. Moreover it is known that P- and E-selectin are present on the endothelium activated by pro-inflammatory stimuli.

It has been previously demonstrated that the treatment with diclofenac is capable to inhibit the expression of E-selectin and other adhesion molecules\textsuperscript{36}.

In group B a significant increase of sE-selectin is observed and this may be explained, in part, by the short duration of the pharmacologic treatment (5 days) and, in part by the dose of diclofenac: one hundred mg daily might in fact be ineffective if the administration is started when a chronic inflammatory reaction is fully developed.

We believe that an increase in sE-selectin does not always corresponds to an increased expression of E-selectin on the cell-surface, but it may be correlated with the shedding of the extracellular part of E-selectin from endothelial cells activated a few hours before the start of the treatment. In fact, after the release of E-selectin 3-4 hours are needed to obtain the maximum level of protein expression at the cell surface and several stimuli were found to activate the E-selectin expression\textsuperscript{37}. Our data on diclofenac activity may be, in part, explained by an increase of the proteolytic cleavage of the surface-expressed molecules.

MBT does not provoke any significant modification on sE-selectin levels and it would be interesting if this might be partly due to the decrease of the cytokine (TNF\(\alpha\), IL-1)-inducible E-selectin.
L-selectin shows a significant modification in group A. Since it is known that the number of L-selectin molecules expressed at the leucocyte surface is much lower than the number of circulating L-selectin molecules present in the same volume of blood, a shedding of L-selectin from large numbers of leucocytes is required to produce a significant increase in the serum, which suggests that an increased L-selectin shedding results from a large pool of activated T-lymphocytes.

This shedding may be important to prevent a too strong interaction via L-selectin and in fact it may be a mechanism for limiting leucocyte aggregation and accumulation at the site of inflammation.

Since L-selectin expression on B and T cells was significantly associated with disease activity in inflammatory conditions an increased serum level may indicate that the cells expressing high selectin levels have been partially removed from the joint and recruited into the circulation. Again, this may be seen as an "end stage" mechanism limiting any further migration of leucocytes into the joints.

Both the treatments (MPT and diclofenac) are not effective on sP-selectin values and the previously observed decrease of inflammatory cytokines by MPT does not allow a cytokine induced production of P-selectin and consequently an increase in its serum levels.

The results of our study indicate that MPT and diclofenac are able to influence different adhesion molecules (L-selectin and E-selectin) in OA patients.

Because of the low number of the patients enrolled further studies will be needed to determine whether changes in cell adhesion molecule in the circulation are due to the altered expression or to different cell removal, because integrated protocols of treatment may possibly induce greater modifications of these markers.

Since it is recognized that some therapies such as MPT are safe and effective, their integration with pharmacological treatment may allow to render the safer use of anti-inflammatory drugs (NSAIDs and CSI).

V CONCLUSION

The pathophysiologic phenomena concurring to joint damage are synovial inflammation and cartilage destruction. In this context chronically inflamed joint tissues express a pattern of adhesion molecules that maintain the activation of inflammatory cells. Selectin family of adhesion molecules is involved in leucocytes recruitment to sites of inflammation and tissue damage.

Present study indicates that MPT and diclofenac are able to influence different adhesion molecules in OA patients. The combination of these two treatments may constitute a safe and effective anti-inflammatory therapy in rheumatic diseases.

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Summary

**Background:** Osteoarthritis (OA) is an important rheumatic condition accompanied by synovial inflammation. Numerous leucocytes are recruited and their migration to the inflamed arthritic joints is mediated by adhesion molecules such as E-, P-, and L-selectins. We measured the serum selectin values in OA patients undergoing Mud Pack Treatment (MPT) or treated with anti-inflammatory drugs to test whether the effect of the treatments may be monitored by the level of serum selectins.

**Materials and Methods:** 50 OA patients were randomly divided into Group A (30 patients undergoing MPT) and Group B (20 patients receiving 50 mg diclofenac twice daily p.o.). Blood samples were collected from both groups before and after the treatments to test serum E-, P-, and L-selectin by ELISA methods.

**Results:** In Group B sE-selectin level showed a significant increment after the drug assumption. In Group A, a significant increment of sL-selectin after MPT was evident, while sP-selectin level did not present any significant variation.

**Discussion:** The study indicates that MPT and diclofenac are able to influence different adhesion molecules in OA patients. The combination of these two treatments may constitute a safe and effective anti-inflammatory therapy in rheumatic diseases.