The effects of nonsteroidal anti-inflammatory drugs on clinical outcomes, synovial fluid cytokine concentration and signal transduction pathways in knee osteoarthritis. A randomized open label trial

L. Gallelli †, O. Galasso ‡*, D. Falcone †, S. Southworth §, M. Greco †, V. Ventura †, P. Romualdi ¶, A. Corigliano †, R. Terracciano †, R. Savino †, E. Gulletta †, G. Gasparini †, G. De Sarro ‡

† Department of Health Science, School of Medicine, Magna Græcia University, Catanzaro, Italy
‡ Department of Orthopedic and Trauma Surgery, School of Medicine, Magna Græcia University, Catanzaro, Italy
§ North Mississippi Sports Medicine & Orthopedic Clinic, PLLC, Tupelo, MS, USA
¶ Department of Pharmacology and Biotechnology, Bologna University, Bologna, Italy

ARTICLE INFO

Article history:
Received 13 January 2013
Accepted 27 June 2013

Keywords:
Knee osteoarthritis
NSAID
WOMAC score
Synovial fluid cytokines
Signal transduction pathways

SUMMARY

Objective: We investigated the effects of celecoxib, diclofenac, and ibuprofen on the disease-specific quality of life, synovial fluid cytokines and signal transduction pathways in symptomatic knee osteoarthritis (OA).

Design: Ninety patients scheduled for a total knee arthroplasty (TKA) were randomized to six groups that were treated with low and high dosages of celecoxib, diclofenac or ibuprofen. At the time of the first admission (T0) and at surgery (T1 = 14 days after beginning of the nonsteroidal anti-inflammatory drugs (NSAIDs)), samples of knee synovial fluid were obtained from each patient for analysis. During the surgery the synovial tissue was harvested from the knee of patients. The Western Ontario and McMaster Universities (WOMAC) score was used to evaluate the patient disease-specific quality of life at T0 and T1. Microarray tests performed at T0 and T1 were used to evaluate the effects of NSAIDs on Tumor necrosis factor (TNF)-alpha, Interleukin-6 (IL-6), IL-8 and Vascular endothelial growth factor (VEGF) concentration in the synovial fluid. Western blot assays evaluated the effects of NSAIDs on MAP kinase (MAPK) signal transduction pathway in the synovial membrane.

Results: NSAID treatment induced a statistically significant improvement in the WOMAC score and a statistically significant decrease in the IL-6, VEGF and TNF-alpha concentration in the synovial fluid. Higher dosages of NSAIDs provided a greater improvement in the disease-specific quality of life of patients and lower concentrations of pro-inflammatory cytokines in the synovial fluid. Inhibition of MAPKs was noted after NSAID treatment.

Conclusion: Short-term NSAID treatment improves the patient disease-specific quality of life with a parallel decrease in pro-inflammatory synovial fluid cytokine levels in knee OA. Signal transduction pathways may be involved in regulating the anti-inflammatory effects of NSAIDs.

ClinicalTrial.gov: NCT01860833.
© 2013 Osteoarthritis Research Society International. Published by Elsevier Ltd. All rights reserved.

Introduction

Central mechanisms may play a role in pain perception during osteoarthritis (OA). However, local inflammation (which involves production of pro-inflammatory cytokines such as interleukin (IL) Tumor necrosis factor (TNF)-alpha, Interleukin-6 (IL-6) and IL-8) is considered to be a major source of pain. In addition to their role in the pathomechanisms of osteoarthritic pain, synovial fluid cytokines...
may in turn contribute to joint destruction and exacerbation of nociception. Several cytokines have been detected in the synovial fluid of patients with early OA. Among these, TNF-alpha and IL-6 were found in 96.1% and 84.6% of OA synovial fluid samples, respectively. TNF-alpha is the major pro-inflammatory cytokine responsible for the shift of cartilage homeostasis towards more catabolism and degradation of the cartilage. In details, it activates sensory neurons directly via its receptors, increases the synthesis of other pro-inflammatory cytokines such as IL-6 and IL-8 and contributes to loss of the cartilage matrix. Indeed, it induces the synthesis of Matrix metalloproteinases (MMPs) and other proteinases by chondrocytes and suppresses cartilage anabolism by inhibiting the synthesis of proteoglycans and type II collagen. IL-6 levels in the synovial fluid are related to the OA stage, and they contribute to cartilage degradation in OA. Moreover, IL-6 could facilitate the excitatory action of substance P on dorsal root ganglion neurons. Vascular endothelial growth factor (VEGF) and its receptors are expressed in OA cartilage and the synovial pannus and are involved in the angiogenesis associated with cartilage destruction during OA. MAP Kinase (MAPK) pathways play a significant role in inflammation and OA. It has been demonstrated that MAPK pathways are able to modulate cytokine-induced chondrocyte responses. Nonsteroidal anti-inflammatory drugs (NSAIDs) have analgesic, antipyretic and anti-inflammatory properties and are extensively prescribed for several musculoskeletal disorders. Indeed, the Osteoarthritis Research Society International (OARSI) recently recommended the use of NSAIDs for management of knee and hip OA in symptomatic patients. These drugs have been shown to influence cytokine metabolism in the synovial fluid of OA patients with satisfactory relief of painful osteoarthritic joints.

The aim of the current study was to explore whether selected NSAID treatment inhibits TNF-alpha, IL-6, IL-8, and VEGF secretion in the synovial fluid of osteoarthritic joints. Diclofenac, ibuprofen and celecoxib were studied. Under the hypothesis that relationships between pro-inflammatory cytokines and the clinical status of OA patients are possible, we also evaluated the association between the concentration of these molecules in the osteoarthritic knee synovial fluid and the pain and functional status of patients with OA. The effects of selected NSAIDs on signal transduction pathways in the synovial membrane were also investigated.

Materials and methods

Study population

From April 2010 to August 2011, 128 patients were evaluated at the Department of Orthopaedic and Trauma Surgery of the Magna Gracia University (Catanzaro, Italy) for primary knee OA and scheduled for a total knee arthroplasty (TKA). Patients eligible for this randomized, open label, parallel-group, single center study should be older than 50 years and should have primary knee OA diagnosed according to the clinical and radiological criteria of the American Rheumatism Association. Further inclusion criteria were clinical signs of joint inflammation (warmth, swelling or effusion) and a disease severity grade 2 or 3 according to the Kellgren–Lawrence (KL) classification. Patients who met the following conditions were excluded: allergy to NSAIDs, progressive serious medical conditions (such as cancer, AIDS or end-stage renal disease), history of gastrointestinal ulcer or bleeding, a hemoglobin concentration lower than 11.5 g/dL, renal diseases (serum creatinine concentration more than 1.2 times the upper limit of the normal range according to the central laboratory definition reference values), or liver dysfunction (serum alanine or aspartate transaminase concentrations more than 1.5 times the upper limit of normal range according to the central laboratory definition reference values). Other exclusion criteria were a diagnosis of rheumatoid arthritis based on the physical examination and laboratory data, alcohol consumption (consuming >3 alcoholic beverages daily) and substance abuse, anticoagulant treatment and inability to give informed consent. Patients were also excluded if they were currently, or within the 3 months prior to inclusion, being treated with corticosteroids or indomethacin. Patients who received intra-articular treatment with corticosteroids or hyaluronic acid within 6 months preceding the study or systemic NSAIDs within 15 days before the study were also excluded. Finally, patients with other painful conditions, or on medication that could possibly confound the evaluation of pain relief (i.e., opioids, paracetamol, corticosteroids, antiepileptics, benzodiazepines and antidepressants), were also excluded.

This study was approved by the Researchers Ethics Committee (number 2010.29) and was conducted in accordance with the Declaration of Helsinki and the Guideline for Good Clinical Practice. All of the participants signed written informed consent prior to enrollment.

Experimental protocol

In this study the eligible patients were randomized into six study groups for treatment (all of the groups consisted of 15 patients), and for 2 weeks an oral dose of the following NSAIDs were given: diclofenac slow release 75 mg/once day or 75 mg/bid (Novartis Pharmaceuticals UK Ltd, Camberley, UK); ibuprofen 600 mg/bid or 600 mg/tid (Abbott laboratories SPA, Campoverde Latina, Italy); or celecoxib 200 mg/once day or 200 mg/bid (Pfizer Inc, New York, NY, USA) (Fig. 1). The patients were allocated using a randomized list produced by a computer-generated table in order to ensure that there were no relevant differences among the study groups with respect to age, sex, OA stage and The Western Ontario and McMaster universities (WOMAC) score.

Clinical assessment

WOMAC osteoarthritis index score was used to measure the disease-specific health status of patients before and after the pharmacological treatment. The WOMAC is a self-administered validated outcome measure that evaluates pain (five items), stiffness (two items), and physical function (17 items). A total WOMAC summary score is calculated for each individual, adjusted, and reported on a 0–100 scale. Lower scores are associated with less pain and stiffness and better function. The safety of the study medications was assessed by monitoring for any adverse drug reactions (ADRs), which were assessed for severity and causality. The severity of ADRs was assessed by a modified Hartwig and Siegel scale that classifies the severity of an ADR as mild, moderate or severe with various levels according to factors such as requirement for change in treatment, duration of hospital stay, and any disability produced by the ADR. To evaluate the relationship between ADRs and drug treatment, the Naranjo adverse probability scale was applied. The Naranjo scale consists of objective questions with three types of responses (yes, no or do not know). The scores are given accordingly and the drug reactions are classified as definite, probable or possible.

The number, duration, and severity of pain attacks, analgesic intake and the occurrence of adverse events were recorded in a daily diary card 1 week prior to the start of the trial and up to 7 days after the surgery. The total blood loss related to TKA was evaluated as previously described. The overall clinical response during the study was assessed by physicians who were blinded to the treatment.
Cytokine evaluation

At the time of the first admission (T0) and at surgery (T1 = 14 days after beginning of the treatment), samples of synovial fluid were obtained from the knee joint of patients for analysis. The fluid samples were aspirated directly or obtained by lavage, corrected for dilution by the urea method and analyzed while blinded to the clinical information.

The synovial fluid was evaluated by the Biochip Array using dedicated evidence instrumentation and software (Randox Laboratories, Crumlin, UK) for simultaneous quantitative detection of IL-6, IL-8, VEGF, and TNF-alpha in a 100 μl sample (supernatant). The Biochip Array is a solid-state device containing arrays of discrete test regions of immobilized antibodies specific to different cytokines and growth factors. The immune reaction is quantitated by chemiluminescence. Increased levels of cytokine are directly related to the signal emitted. Digital imaging technology is used to detect the signal and the concentration of each analyte is calculated from the calibration curve. Cytokines in the supernatant were detected as pg/ml. The essay ranges were 0–707 pg/ml for the TNF-alpha, 0–1063 pg/ml for IL-6, 0–1492 pg/ml for IL-8, and 0–3043 pg/ml for VEGF. Each experiment was performed in triplicate. All of the samples were evaluated by an operator who was blinded to the experimental design.

Signal transduction pathways

A 2 cm² sample of the synovial membrane was taken from each patient treated with NSAIDS at the time of surgery. A small control group of patients (n = 10) with knee OA who were subjected to TKA were also enrolled in the study. These patients fulfilled the same inclusion and exclusion criteria of the patients randomized into the groups of treatment and they did not receive any NSAID treatment before surgery. The control group did not differ for sex, age and OA stage in comparison with the groups of treatment (Table I). Each synovial membrane was lysed in Thermo Scientific T-PER Tissue Protein Extraction Reagent (Thermo Scientific Inc, Waltham, MA, USA) that was enriched with both protease and phosphatase inhibitors. The protein extracts were then separated as previously described.

Immunoblotting was performed using anti-phospho-c-Jun N-terminal kinases (JNK), anti-phospho-p38, anti-phospho-extracellular signal-regulated kinases (ERK)1/2, and anti-phospho-RAS monoclonal antibodies (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) as previously described. To normalize for protein loading, after being stripped the membranes that were probed with anti-phospho-ERK1/2 were re-probed with polyclonal antibodies against total (phosphorylated and unphosphorylated) ERK1/2 (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA). The membranes probed with anti-phospho-JNK, anti-phospho-p38, and anti-phospho-RAS monoclonal antibodies were re-probed with an anti γ-tubulin monoclonal antibody (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA). The blots were also incubated with a mouse monoclonal caspase-3 (E-8) antibody, directed against both inactive pro-caspase-3 and active caspase-3, a common enzymatic marker of apoptosis. Again, in order to normalize for protein loading, the membranes were re-probed with an anti γ-tubulin

### Table I

<table>
<thead>
<tr>
<th>Sex</th>
<th>Ibuprofen 1200 mg/day</th>
<th>Ibuprofen 1800 mg/day</th>
<th>Diclofenac 75 mg/day</th>
<th>Diclofenac 150 mg/day</th>
<th>Celecoxib 200 mg/day</th>
<th>Celecoxib 400 mg/day</th>
<th>Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>7</td>
<td>11</td>
<td>9</td>
<td>6</td>
<td>0.596</td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>8</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>0.094</td>
</tr>
<tr>
<td>Age</td>
<td>67.3 ± 4.9 (55–73)</td>
<td>66.1 ± 6.8 (53–78)</td>
<td>68.4 ± 3.6 (63–75)</td>
<td>67.3 ± 4.8 (59–78)</td>
<td>69.1 ± 6.3 (59–81)</td>
<td>63.5 ± 4 (57–72)</td>
<td>68 ± 4.8 (63–74)</td>
<td>0.094</td>
</tr>
<tr>
<td>OA stage</td>
<td>2</td>
<td>7</td>
<td>6</td>
<td>8</td>
<td>9</td>
<td>5</td>
<td>7</td>
<td>0.606</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>6</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>10</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>T0 WOMAC</td>
<td>60 ± 8.8 (47–65)</td>
<td>60.9 ± 7.8 (46–77)</td>
<td>62.1 ± 6.8 (43–69)</td>
<td>59.3 ± 6.9 (45–71)</td>
<td>60.2 ± 8.6 (45–78)</td>
<td>62.4 ± 7.4 (49–72)</td>
<td>n.a.</td>
<td>0.865</td>
</tr>
</tbody>
</table>

Fig. 1. Schematic representation of treatment groups.
monoclonal antibody (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA). Antibody binding was visualized by enhanced chemiluminescence (ECL-Plus, Amersham Pharmacia Biotech, Piscataway, NJ, USA). The intensities of the experimental bands were analyzed by computer-assisted densitometry as previously described\(^{29}\) and expressed as arbitrary units (AU).

All of these experiments were performed in triplicate by an operator who was blinded to the experimental design.

**Sample size and statistical analysis**

The primary outcome for the power calculation was an improvement in the WOMAC score.

As previously described, a 17% WOMAC score difference was considered to be the minimal clinical improvement threshold that was important from the subject’s perspective\(^{30}\). To detect a clinically relevant difference between each group 90 subjects are needed (power >85%, alpha 0.05, two-tailed). Regarding the effects on the synovial concentration of cytokines, we were unable to define a clinically significant reduction or to determine a power calculation. In this light, these results could only be labeled as exploratory.

The mean, standard deviation and range were reported for the continuous variables, whereas the counts described the categorical variables. Data were checked for normality using the Kolmogorov-Smirnov test. The Student’s \(t\) test and the \(\chi^2\) test were used when appropriate to test the significance of the differences. The analysis of variance (ANOVA) was used to evaluate the differences between multiple means. Once we determined that differences existed, a Bonferroni test was used to determine which means differed. An age-adjusted univariate linear and logistic regression analysis was used to evaluate the relationship between the explanatory variables and the outcomes. Explanatory and confounding variables included in the analysis were: age (continuous), gender (categorical), radiological OA classification (discrete), body mass index (BMI) (continuous), baseline WOMAC score (continuous), baseline cytokine concentration (continuous), WOMAC score at follow-up (continuous), WOMAC score variation (continuous), baseline cytokine concentration (continuous), cytokine concentration at follow-up (continuous), variation of cytokine concentration (continuous), and ADRs (continuous) were treated as outcomes and the effect of possible predictors was checked for their value. A \(P\) value of less than 0.05 was considered statistically significant. The SPSS (SPSS Inc., Chicago, USA) and G*Power (Institut für Experimentelle Psychologie, Heinrich Heine Universität, Dusseldorf, Germany) software were used for the statistical analyses.

**Results**

**Disease-specific quality of life**

After a detailed clinical history and radiological examination, 90 patients, 54 women (67.2 \(\pm\) 5.1 years) and 36 men (66.31 \(\pm\) 4.8 years), with a mean body mass index of 34.5 \(\pm\) 3.7 (men 33.5 \(\pm\) 3.52; women 34.4 \(\pm\) 3.9) were recruited in this trial. Patients’ data are shown in Table I.

The differences in the WOMAC index at T0 were not statistically significant between the groups. No patients were lost to follow-up and the WOMAC score variation after NSAID treatment for the groups of patients is shown in Fig. 2. All of the NSAIDs improved the WOMAC scores, and the degree of improvement was greater at higher dosages of drugs (\(P < 0.001\)). Ibuprofen 1800 mg/day exhibited a higher WOMAC score improvement with respect to celecoxib 200 mg (95% CI: 5.8–8.8, \(P < 0.001\)), but not compared to celecoxib 400 mg/day (95% CI: −1.04 to 2.84, \(P = 0.351\)). The effects of celecoxib 400 mg on the WOMAC score variation was significantly higher in comparison to diclofenac 150 mg (95% CI: 0.15–4.49, \(P = 0.037\)); conversely, no significant differences were noted between ibuprofen 1800 mg and diclofenac 150 mg (95% CI: −0.43 to 2.83, \(P = 0.143\)). The effects of each NSAID were independent of the age of enrolled patients. In all but two groups the WOMAC score variation did not differ according to gender (Supplementary data).

**Synovial fluid cytokines**

Synovial fluid samples were obtained from all of the patients in the study. No out of range values were found. On the whole, NSAID treatment induced a dose-dependent reduction of pro-inflammatory cytokines without differences between the different NSAIDs. Higher dosages of diclofenac, ibuprofen and celecoxib were associated with a greater reduction of IL-6, VEGF and TNF-alpha synovial fluid concentrations (Figs. 3–5) in comparison to the lower dosages. NSAID treatment did not significantly modify IL-8 synovial concentration with any drug at any dose (data not shown). In the whole study population of 90 individuals, a direct correlation between the concentration at T1 of most of the molecules evaluated in the synovial fluid and an improvement in the total WOMAC was noted (WOMAC vs IL-6: \(\beta = 0.914, P = 0.005\); WOMAC vs VEGF: \(\beta = 0.985, P = 0.008\); WOMAC vs TNF-alpha: \(\beta = 0.975, P < 0.001\)). No statistically significant correlations were noted between the concentration of cytokines or VEGF at T0 or their

---

**Fig. 2.** Effects of NSAIDs on WOMAC score. All values are expressed means ± standard deviation for each treatment group (\(n = 15\)) and were evaluated as difference between the values at T1 and T0.
variation after NSAID treatment and the radiographic findings for the knee joint (KL grade 2 vs KL grade 3, Supplementary data).

Signal transduction pathways

The effect of NSAID on signal transduction pathways was tested only at the highest dosage. NSAID treatment induced a statistically significant ($P < 0.01$) inhibition of ERK, JNK, p38 and RAS phosphorylation (Fig. 6) along with caspase-3 activation (Fig. 7) with respect to the control patients.

NSAID safety

A higher postoperative blood loss was recorded in two women treated with ibuprofen (1800 mg/day) (mean blood loss: $945 \pm 115$ mL) and in two women and one man treated with diclofenac (150 mg/day) (mean blood loss: $915 \pm 120$ mL) in comparison to the average values of the remaining patients (mean blood loss: $580 \pm 270$ mL). Two further other patients treated with ibuprofen 1800 mg/day experienced nausea 4 days after beginning the drug. The ADRs were categorized as probable based on the modified Hartwig and Siegel scale of severity assessment, and the reactions were categorized as “mild level 1” severity.

Discussion

The major aims in OA management are to provide pain relief and to facilitate rehabilitation and return to normal function$^{31,32}$. One possible therapeutic strategy may be inhibition of inflammatory activity in the synovial tissue through NSAID administration$^{17}$. In this study we have shown that diclofenac, ibuprofen and celecoxib used for OA treatment decrease IL-6, TNF-alpha and VEGF in the synovial fluid of the osteoarthritic joint with a parallel improvement in joint pain and function of patients. This is the first study looking at the effects of diclofenac and ibuprofen on the synovial fluid levels of IL-6 in patients with knee OA. In a previous study, after 14 days of treatment with celecoxib or nimesulide a significant reduction in the IL-6 levels was observed in the synovial fluid of patients with knee OA$^{17}$. Similarly, Schumacher et al.$^{18}$ demonstrated that IL-6 levels decrease in the synovial fluid of osteoarthritic patients after two weeks of oral etodolac. The NSAID-induced reduction of the IL-6 concentration may be explained at least in part by inhibition of prostaglandin (PG) production by NSAIDs. Indeed, even though some of the NSAIDs developed in recent years show greater activity against cyclooxygenase (COX)-2, all NSAIDs inhibit the different isoforms of COX. These enzymes promote the synthesis of PGE2 and other prostaglandins through the conversion of arachidonic acid. It has been previously demonstrated that prostaglandins stimulate the production of IL-6 by bone cells$^{19}$. This hypothesis is in agreement with the observation that OA patients treated with celecoxib exhibit decreased IL production in the gastric mucosa$^{20}$. Therefore, the effect of NSAIDs on the IL-6 concentration is unlikely to be tissue specific. Interestingly, it has been previously reported that celecoxib is able to decrease TNF-alpha and PGE2 in the synovial membrane and fluid, respectively, after a 3-month treatment in patients with knee OA$^{18}$. These
data concur with this study. We are not aware of any study looking at the effect of NSAID treatment on the VEGF synovial fluid concentration. The current study demonstrates that all of the tested NSAIDs are able to decrease VEGF synovial fluid levels in patients with OA. Notably, it has been previously documented that VEGF expression by the chondrocytes is increased in OA, and these levels correlate with the degree of OA\textsuperscript{17}. In agreement with previous findings\textsuperscript{17}, in the current study no tested NSAID affected IL-8

![Fig. 5.](image1)

![Fig. 6.](image2)

Fig. 5. TNF-alpha concentrations in synovial fluid before (T0) and after NSAID treatment for 14 days (T1) (n = 15) for each group.

Fig. 6. Effects of celecoxib (400 mg/day), diclofenac (150 mg/day) and ibuprofen (1800 mg/day) for 14 days on JNK (panel A), p38 (panel B), ERK (panel C), and RAS (panel D) phosphorylation in enrolled patients compared to control patients. Densitometric evaluation of western blots is expressed as arbitrary units. Values are means ± standard deviation, n = 15 in each NSAID group; n = 10 in control group.
concentrations in the synovial fluid at any time, suggesting that secretion of this cytokine during OA is likely to be regulated by different pathways.

Unsurprisingly, we documented a greater reduction in the IL-6, VEGF and TNF-alpha levels after treatment with higher NSAID dosages, in particular with ibuprofen and celecoxib. Classical NSAIDs have been correlated with gastrointestinal, cardiovascular and renal events. We only observed increased bleeding in some of the patients treated with ibuprofen and diclofenac at higher dosages. The absence of serious ADRs in our study could be related to several factors such as the short treatment time (14 days) and the exclusion of patients with a history of gastrointestinal, cardiovascular, renal and liver diseases.

In the current study, the NSAID treatment induced a statistically significant inhibition of ERK, JNK, p38 and RAS phosphorylation along with caspase-3 activation in the synovial membrane of OA patients with respect to the controls. As far as we know, this effect has never been described previously. Indeed, it was demonstrated that NSAIDs inactivate MAPKs in the articular cartilage but their effect on the synovial membrane was not evaluated. The possibility that the inhibition of MAPKs results from the decreased levels of cytokines induced by NSAID treatment cannot be ruled out. Indeed, it was reported in a transgenic mouse model that TNF-enhances chronic inflammation of the synovial membrane by activation of MAPK cascade. It is tempting to speculate that the high levels of MAPK activation we have observed in control patients influence the progression of OA and that the inhibition of MAPKs after NSAID treatment (Fig. 6) correlates with the effects of NSAIDs on WOMAC score (Fig. 2). Indeed, MAPK activity plays a key role in the regulation of MMPs production by chondrocytes and it may contribute to the development of OA through down regulation of peroxisome proliferators-activated receptor gamma and up regulation of inducible NO synthase.

We next demonstrated that the WOMAC scores significantly improved after diclofenac, celecoxib or ibuprofen treatment. This is in agreement with recent papers showing that celecoxib and ibuprofen are able to improve WOMAC scores in patients with knee OA. This effect could be related to the ability of NSAID treatment to reduce synovitis and joint effusion due to degenerative changes in the osteoarthritic joint. Interestingly, a significant correlation between the plasma CRP levels and synovial fluid IL-6 levels or the degree of synovial inflammatory infiltration has been documented in patients with knee OA. A direct association between improvement in the total WOMAC score and the decrease in the cytokine concentration at T1 was reported in the current study. Apart from a correlation between PGE2 and the WOMAC index, Brenner et al. failed to find any significant relationships between IL-6 or TNF-alpha and any of the assessed clinical signs. On the contrary, and in agreement with our findings, a significant positive correlation between the total WOMAC score and its subscales with TNF-alpha in patients with OA of the knee was previously reported. The authors noted a similar correlation between IL-6 and the WOMAC stiffness domain.

Some weaknesses of the current study design might limit the conclusions that can be drawn from this data. We were not able to examine normal knees to evaluate both the cytokine levels in the synovial fluid and MAPK activation in the synovial membrane. However, there are obvious ethical concerns in obtaining control synovial fluid and synovial membrane from intact knee joints. Further limitations of the current study have to be acknowledged. First, the cytokine gene expression was not evaluated and variations in these genes have been associated with different levels of inflammation. Second, we only examined the synovial fluid and not the serum levels of the cytokines. Thus, we could not report any correlation between the serum and synovial fluid levels of these molecules. Finally, only OA patients with evidence of active joint inflammation were included in this trial. Hence, we cannot be confident that our result is applicable beyond this phenotype. However, despite some so-called noninflammatory types of OA are usually identified, inflammation is directly responsible for several clinical symptoms and reflects the progression of the disease.

In the current study, the question concerning the influence of NSAIDs on the progression of structural changes in OA remains unanswered. An association between synovial inflammatory processes and OA progression has been reported, thus supporting the use of these drugs to treat OA. However, certain studies suggest that some NSAIDs inhibit matrix synthesis by articular cartilage in vitro. There are also reports of increased rate of OA progression...
in patients receiving NSAIDs. In this light, long-term NSAID treatments should be discouraged in patients with OA. However, the controversial effects of prolonged NSAID treatment in OA overpasses the issue of this study, which evaluated the effects of this therapy after a short-term period.

In conclusion, the current study suggests that short-term treatment with oral celecoxib, ibuprofen and diclofenac effectively suppress proinflammatory cytokines and improves pain and function in patients with symptomatic knee OA without serious adverse events. Higher dosages of the evaluated NSAIDs provide better improvement in the disease-specific quality of life of OA patients and a greater modulation of cytokine secretion. Further studies looking at molecular pathways implicated in the NSAID response during OA are warranted.

Contributions

LG and OG conceived the study, had full access to all of the data, and wrote the manuscript. AC and GG acquired the data. DF, MG, VV, RT, EG and RS performed most of the experiments. LG performed the statistical analyses. SS, PR and GDS made a critical revision of the manuscript for important intellectual content. All authors have read and approved the final manuscript.

Competing interest statement

Authors declare no conflict of interests.

Acknowledgments

Daniela Falcone was supported by a fellowship from the PhD Programme in Molecular Oncology, Experimental Immunology and Development of Innovative Therapies. We are grateful to Curto Lucia Stella for skilled experimental support and to Marco De Gori, M.D., for his statistical advice.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.joca.2013.06.026.

References


