EFFECTS OF PIROXICAM, TENOXICAM AND MELOXICAM ON NITRIC OXIDE LEVELS IN PATIENTS WITH OSTEOARTHRITIS

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Summary
The aim of this study was to compare the effects of three non-steroidal anti-inflammatory drugs (NSAIDs) belonging to the oxicam group: piroxicam, tenoxicam and meloxicam on nitric oxide level as a marker of oxidative stress in patients with knee osteoarthritis. Twenty-eight adult patients clinically and radiographically diagnosed with knee osteoarthritis previously untreated were enrolled. The serum levels of nitric oxide were assessed at baseline and after 20 days of treatment with: piroxicam at a dose of 20 mg po daily, tenoxicam at a dose of 20 mg po daily, and meloxicam, 15 mg po daily. Piroxicam treated patients had a significant decrease in nitric oxide levels, those treated with tenoxicam had no significant change, whereas meloxicam treated patients had unchanged nitric oxide levels. Our study revealed that piroxicam had a significant nitric oxide lowering effect in patients with knee osteoarthritis, indicating interferences in nitric oxide pathways.

Key words: osteoarthritis, piroxicam, tenoxicam, meloxicam, oxidative stress, nitric oxide.

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Introduction
Osteoarthritis (OA), a major public health problem that ranks second among chronic conditions after the cardiovascular diseases (Rosu & Vreju, 2007) is defined as a heterogeneous group of diseases that cause joint manifestations associated with alterations in the integrity of cartilage, accompanied by changes in subchondral bone and periarticular structures (capsule, ligaments, tendons, muscles).

OA incidence is increasing mainly due to higher life expectancy and has significant consequences in society (Sarzi-Puttini et al., 2005; Rosu & Vreju 2007). Changes in the articular cartilage affected by OA come from the imbalance between anabolic and catabolic processes influenced by biomechanical forces and defective autocrine, paracrine and endocrine regulation at cellular level, resulting in an abnormal turnover of articular tissue (Herrero-Beaumont et al., 2009). Formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) has been suggested to play significant roles in various inflammatory diseases such as rheumatoid arthritis, ankylosing spondylitis and OA (Ozgocmen et al., 2005).

There are many studies that have focused on the destructive effects of oxidative and nitrosative agents in the pathogenesis of OA. Oxidative stress is involved in cartilage destruction in OA (Ziskoven et al., 2010) and also has indirect action by activating collagenases and up-regulating genes encoding enzymes involved in matrix degradation and cytokines production (Rees et al., 2008).
Maneesh et al. showed a higher level of oxidative stress in patients with OA compared with healthy patients (Maneesh et al., 2005).

Due to an increased formation of ROS, results a higher possibility of interaction between nitric oxide (NO) and superoxide, leading to RNS responsible for indirect effects, such us nitrosylation, nitrification or oxidation of biomolecules (Feelisch, 2008).

NO is formed by conversion of L-arginine to L-citruline. The reaction is catalyzed by nitric oxide synthase (NOS) which oxidises one of the guanidinil nitrogens of arginine to anhydroxy arginine which is further oxidised to citruline and NO (Abramson, 2008). There are three NOS isoforms: neuronal (nNOS sau NOS I), endothelial (eNOS sau NOS III) and inducible (iNOS sau NOS II) (Scher et al., 2007).

Inflammation and mechanical stress are associated with up-regulation of NO (Ziskoven et al., 2010), which may have adverse effects on chondrocytes, such as inhibition of collagen and proteoglycans synthesis, modulation of cytokines expression, matrix metalloproteinases (MMP) activation (Fermor et al., 2007). NO is an important promoter of chondrocytes catabolic activity, inhibiting cartilage matrix synthesis, accelerating chondrocyte-mediated matrix degradation, promoting chondrocyte apoptosis and inflammatory responses, leading to loss of cartilage matrix (Scher et al., 2007).

OA progression is correlated with cell senescence and apoptosis in all joint compartments, ROS/RNS being involved in these processes by reducing the ability of chondrocytes to maintain and repair cartilage (Ziskoven et al., 2010, Starodubtseva, 2011).

NO and other free radicals lead to cell death and promote protein nitrosylation (Chevalier, 2007). Nitrotyrosine formed by oxidation of tyrosine in the presence of NO is overexpressed in OA cartilage and in aging cartilage (Afonso et al., 2007).

Thus, in pathological circumstances, NO and ROS contribute to cartilage destruction through direct degradation of matrix components, increasing catabolic cytokines activity and reducing cartilage repair ability (Henrontin et al., 2005).

According with OARSI recommendations (Osteoarthritis Research Society International), cyclooxygenase (COX) non-selective and selective oral NSAIDs are included among pharmacological treatment strategies for OA (Zhang et al., 2008). NSAIDs influence prostaglandin synthesis through inhibition of COX. There are two COX isoforms that have been well-recognized: COX-1 and COX-2. COX-1 is a constitutively expressed isofrom and tends to have a homeostatic function, whereas COX-2 is inducible during inflammation and facilitate the inflammatory response. Non-selective COX inhibitors may determine side effects (gastrointestinal damage (GI) and platelet dysfunction), while selective COX-2 inhibitors reduce inflammation with fewer GI side effects (Rusu&Vreju, 2005). NSAIDs affect ROS formation, some attenuate whereas others enhance ROS generation.

Our aim was to compare the in vivo effect of meloxicam, a selective COX-2 inhibitor on NO levels, with that of piroxicam and tenoxicam, non-selective COX inhibitors.

**Material and methods**

**Patients**

Patients with OA of the knee enrolled in this study were recruited from the Medical Clinic No. 1, Emergency County Hospital from Craiova. Distribution of patients in groups was made according to a study protocol approved by the Ethics Committee of University of Medicine and Pharmacy of Craiova. 28 patients with OA of the knee were selected to assess blood NO levels at baseline and after 20 days of treatment with piroxicam, tenoxicam or meloxicam; 10 patients were treated with piroxicam, 20 mg po daily, 10 patients with...
tenoxicam, 20 mg po daily and the rest with meloxicam, 15 mg po daily. Piroxicam-treated patients (2 men, 8 women) had a mean age of 52.8 ± 4.23 yr (range 46-61). Tenoxicam treated patients (2 men, 8 women) had a mean age of 54.2 ± 2.15 yr (range 50-57). Meloxicam treated patients (2 men, 6 women) had a mean age of 61 ± 10.02 yr (range 51-73).

**Inclusion criteria.** Patients aged ≥18 years old with primary OA of the knee, diagnosed using clinical and radiographic assessment with American College of Rheumatology criteria (Altman et al., 1986) were recruited for the study. All the patients voluntarily participated in the study and gave their informed consent.

**Exclusion criteria.** Patients with the following conditions were excluded from the study: current drug usage for uncontrolled concomitant disease or chronic conditions that might interfere with the assessment of clinical findings of OA (inflammatory arthritis, gout or Paget’s disease) and serum oxidative stress markers (diabetes or other metabolic diseases, allergies, asthma, Parkinson’s disease, atherosclerosis, inflammatory bowel disease, hematopoietic disorders, hypertension); a history of gastrointestinal ulcers and bleeding; diagnosis of chronic pain syndrome (e.g., fibromyalgia, chronic fatigue syndrome); excessive alcohol consumption, smoking and regular aerobic exercise program; intramuscular, intravenous or soft tissue injection of corticosteroids within 1 month prior to the study; use of intraarticular corticosteroids within 2 months prior to the study; history of clinically significant intolerance to oxicams. Patients who had hepatic dysfunctions, renal dysfunctions or infectious disease were excluded. Treatment with statins, other NSAIDs, enzyme-inducing drugs or enzyme-inhibiting drugs was not allowed. No supplementary therapies, special diets or aerobic exercise programs were allowed during the study period.

**Biochemical evaluation.** Venous blood samples were collected à jeun at baseline and after 20 days for analysis of NO level. The blood was processed to obtain plasma and the supernatant was frozen until use for analysis. Since NO is very labile, its direct measurement in the biological samples is difficult. In aqueous solution, NO reacts with oxygen and accumulates in the plasma as nitrite (NO$_2^-$) and nitrate (NO$_3^-$) ions. These products are stable, can be readily measured in biological samples and are used as indicators of NO production in vitro and in vivo. The plasma total nitrite concentration is accepted as an index of NO production. For total nitrite detection, plasma was assessed by a colorimetric assay based on the Griess reaction (BioVision, Nitric Oxide Colorimetric Assay Kit). Briefly, a chromophore with a strong absorbance at 540 nm is formed after the reaction of nitrite with sulphanilamide and N-naphthylethylenediamine in a 96-wells plate. A standard curve is established with a set of serial dilutions of sodium nitrite and results are expressed as μmol nitrites/L of plasma.

**Statistical analysis.** Statistics Package for Social Sciences was used for the statistical analysis. Results were expressed as mean ± SD. Differences between the three groups at baseline were assessed by independent sample t test. Changes observed before and after oxicams treatment were assessed by the paired sample t test. A p value of <0.05 was considered statistically significant.

**Results**

All patients enrolled completed the study. The baseline NO levels in piroxicam and tenoxicam groups had no significant difference when compared to each other. In the tenoxicam group, baseline NO levels were slightly higher than in the other two groups (Table 1). Piroxicam-treated patients had a significant decrease in NO levels after 20 days, those treated with tenoxicam had no significant change in NO levels, while meloxicam-treated patients had unchanged NO levels (Table 1, Figure 1).
Table 1. Plasma nitrite levels, as an index of NO, in oxicam treated groups

<table>
<thead>
<tr>
<th>Oxicam-treated group</th>
<th>Age (mean±SD)</th>
<th>Nitrite (μmol/L) Baseline (mean±SD)</th>
<th>Nitrite (μmol/L) Final (mean±SD)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piroxicam</td>
<td>52.8±4.23</td>
<td>21.57±6.86</td>
<td>17.68±4.29</td>
<td>0.006</td>
</tr>
<tr>
<td>Tenoxicam</td>
<td>54.2±2.15</td>
<td>30.71±13.88</td>
<td>23.88±7.29</td>
<td>0.213</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>61±10.02</td>
<td>21.02±8.29</td>
<td>20.60±9.82</td>
<td>0.915</td>
</tr>
</tbody>
</table>

*p values represent baseline versus final measurements in each treatment group (paired t test)

Discussion

OA cartilage produces large amounts of NO and ROS. Chondrocytes iNOS is up-regulated in osteoarthritis which increases NO synthesis and IL-1 and TNFα stimulate NO production in cartilage (Poole, 1999). NO can react with superoxide anions (O$_2^•−$) to generate peroxynitrite, which is involved in proinflammatory and proapoptotic effects on cartilage (Abramson, 2008).

NO is involved in the promotion of OA cartilage catabolism through several mechanisms: inhibition of synthesis of cartilage matrix macromolecules (e.g. aggregan (Poole, 1999), increased MMP activity (Martel-Pelletier et al., 2008), reducing synthesis of IL-1Ra (IL-1 receptor antagonist), an endogenous inhibitor of IL-1β (Scher et al., 2007). NO is involved in chondrocyte apoptosis (Poole, 1999) and induces COX-2 activity and PGE2 synthesis (Martel-Pelletier et al., 2008), but inhibition of PGE2 by COX-2 inhibitors do not affect spontaneous NO production significantly (Lotz, 1999). In turn, PGE2 can sensitize OA chondrocytes to NO-induced cell death. NO acts through reduction of major anabolic processes and increase of catabolic reactions (Martel-Pelletier et al., 2008).

NSAIDs effects on oxidative stress markers in OA patients was assessed in relatively few studies.

Van Antwerpen and Nève investigated *in vitro* anti-oxidizing effects of some NSAIDs belonging to the oxicam group, ibuprofen and nimesulide, and discovered that oxicams are more reactive against ROS than nimesulide and ibuprofen (Van Antwerpen & Nève, 2004). Bartosiewicz et al. have determined that serum levels of MDA and serum oxidative capacity decreased in patients with OA.
treated with piroxicam (Bartosiewicz et al., 1993). Tuzun et al. showed that tiaprofenic acid and flurbiprofen influence serum levels of NO, MDA, and SOD (Tuzun et al., 2005). Cimen et al. examined the in vivo effect of celecoxib, ibuprofen and tenoxicam on free radical metabolism in erythrocyte of OA patients, finding a similar influence on antioxidant potentials and SOD for all three drugs, despite their different mechanisms of action on COX (Cimen et al., 2003). Ozgocmen et al. show that tenoxicam may have antioxidant effects, and celecoxib and tenoxicam may reduce nitrite levels (Ozgocmen et al., 2005). Yoon et al. showed that NSAIDs inhibit NO-induced apoptosis independent of their effects on COX activity (Yoon et al., 2003).

Our findings – a significant NO lowering effect for piroxicam (a nonselective COX inhibitor), but an insignificant effect of tenoxicam (also a nonselective NSAID inhibitor) and of meloxicam (a selective COX-2 inhibitor) are contradictory.

Conclusions

The study of the effects of 20 days treatment with piroxicam, tenoxicam and meloxicam in patients with knee osteoarthritis showed that tenoxicam and meloxicam had no significant effect on NO levels, whereas piroxicam reduced its plasma level, indicating interferences in nitric oxide pathways.

Further studies are required to assess the NSAIDs effects on NO pathways since those mechanisms might provide new therapeutic approaches for prevention of cartilage damage and OA progression.

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