

BLOOD SUPEROXIDE DISMUTASE ACTIVITY IN PATIENTS WITH KNEE OSTEOARTHRITIS TREATED WITH OXICAMS

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Abstract

The aim of this study was to compare the effects of three non-steroidal anti-inflammatory drugs (NSAIDs) belonging to the oxicam class: piroxicam and tenoxicam, as nonselective inhibitors of cyclooxygenase (COX), and meloxicam, a selective COX-2 inhibitor, on superoxide dismutase (SOD) activity in patients with knee osteoarthritis. Thirty adult patients clinically and radiographically diagnosed with knee osteoarthritis previously untreated were enrolled and divided in three groups. The serum levels of SOD were assessed at baseline and after 20 days of treatment with: piroxicam at a dose of 20 mg p.o. daily, tenoxicam at a dose of 20 mg p.o. daily, and meloxicam 15 mg p.o. daily. Piroxicam treated group had a significant increase in SOD activity ($p = 0.04$), meloxicam treated group had no significant change in SOD activity ($p = 0.11$), whereas tenoxicam treated group had unchanged SOD activity ($p = 0.85$). The study of the effects of 20 days treatment with NSAIDs belonging to oxicam group in patients with knee osteoarthritis revealed that piroxicam significantly increase SOD activity.

Rezumat

Scopul acestui studiu a fost compararea efectelor a trei medicamente antiinflamatoare nesteroidiene din clasa oxicamilor – piroxicamul și tenoxicamul ca inhibitori neselectivi ai COX și meloxicamul, un inhibitor selectiv al COX-2 – asupra activității serice a enzimei superoxid dismutază (SOD) la pacienți cu gonartroză. În studiu au fost incluși treizeci de pacienți adulți, împărțiți în trei loturi, diagnosticați clinic și radiografic cu gonartroză, dar care nu au mai fost tratați în prealabil pentru gonartroză. Nivelul seric al SOD a fost determinat la începutul și după 20 de zile de tratament cu piroxicam în doze de 20 mg p.o. zilnic, tenoxicam în doze de 20 mg p.o. zilnic, respectiv cu meloxicam în doze de 15 mg p.o. zilnic. În cazul lotului tratat cu piroxicam s-a constatat o creștere semnificativă statistic a activității SOD ($p = 0.04$), în timp ce la lotul tratat cu meloxicam nu au existat modificări semnificative ($p = 0.11$), iar în cazul lotului tratat cu tenoxicam activitatea SOD nu s-a modificat ($p = 0.85$). Studiul tratamentului timp de 20 de

zile cu oxicami la pacienți cu gonartroză a arătat că piroxicamul determină creșterea semnificativă a activității SOD.

Keywords: superoxid dismutase (SOD), oxidative stress, osteoarthritis, oxicams.

Introduction

Osteoarthritis (OA) is a heterogeneous group of diseases causing joint manifestations, characterized by degeneration and loss of articular cartilage and changes in subchondral bone and periarticular structures [3]. Reactive oxygen and nitrogen species (ROS/RNS) are playing important roles in the pathogenesis of osteoarthritis, a higher level of oxidative stress being observed in OA patients compared with healthy patients [6]. Oxidative stress is implicated in cartilage destruction in OA [16]. Indirectly, oxidative stress is involved in OA pathogenesis by activation of collagenases and up-regulation of genes encoding enzymes implicated in matrix degradation and cytokines production [9, 16].

According with OARSI (Osteoarthritis Research Society International) recommendations, cyclooxygenase (COX) non-selective and selective oral antiinflammatory drugs are included among pharmacological treatment modalities for OA [15]. NSAIDs influence prostaglandin (PG) synthesis through inhibition of COX enzyme. There are two COX isoforms that have been well-characterized: COX-1 and COX-2. In osteoarthritis, NSAIDs act through inhibition of PG synthesis by blocking COX activity, but part of their action may be related to other mechanisms, such as: reduction of superoxide and free radical generation [1], prevention of synthesis and release of free radicals and degradative enzymes of synovial macrophages and polymorphonuclear leukocytes, influencing the mobility, chemotaxis and aggregation of neutrophils and macrophages, but also direct neutralization of free radicals [12].

There are studies that suggest a possible link between oxidative stress and inflammation-mediated induction of COX. Free radicals are generated in PG synthesis therefore PG synthesis inhibitors reduce their production [1]. NSAIDs affect ROS formation, some attenuate whereas others enhance oxidative stress.

According to these considerations numerous studies have been conducted and they have shown the ability of NSAIDs to interfere with oxidative stress in various diseases.

Oxicams represent a NSAIDs class of compounds comprising enolic acid structure and can act as nonselective COX inhibitors (piroxicam, tenoxicam, lornoxicam) and as COX-2 selective inhibitors (meloxicam).

Superoxide dismutase (SOD), glutathione peroxidase, and catalase neutralize ROS, being considered as antioxidant enzymes [11]. Superoxide dismutase is the enzyme that catalyzes the conversion of superoxide anion radicals ($O_2^{\bullet-}$) to hydrogen peroxide and molecular oxygen, functioning as a controller of cellular ROS levels [8].

Our aim was to compare the *in vivo* effect of piroxicam and tenoxicam, as nonselective COX inhibitors, to meloxicam, a selective COX-2 inhibitor, on SOD activity.

Materials and Methods

Patients and sample collection

30 patients with knee osteoarthritis were recruited voluntarily from the Medical Clinic No. 1, Emergency County Clinical Hospital No.1 Craiova after each of them signed an informed consent. The study was conducted in accordance with the WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects being approved by the Ethical Commission of the University of Medicine and Pharmacy of Craiova.

Distribution of patients in groups was made according to well defined inclusion and exclusion criteria. The first group of 10 patients was treated with piroxicam, 20 mg po daily; the second group of 10 patients with tenoxicam, 20 mg po daily and the rest with meloxicam, 15 mg po daily. Blood SOD activity was assessed at baseline and after 20 days of treatment. Venous blood samples were collected *à jeun*, processed for erythrocyte hemolysis and then used for the measurement of SOD activity.

Determination of superoxid dismutase activity

SOD activity in erythrocytes lysate was measured using a commercial kit (Randox Laboratories) based on the generation of superoxide radical through the oxidation of xanthine by xanthine oxidase and its reaction with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The degree of inhibition of this reaction represent SOD activity that was converted to SOD units/ml of whole blood and reported to the normal ranges mentioned for this method (164 – 240 U/mL).

Statistical analysis

For the statistical analysis was used Statistics Package for Social Sciences (SPSS) and results were expressed as mean $\pm$ SD (standard deviation). Differences between the three groups at baseline were assessed by independent sample t test. Changes observed before and after treatment were assessed by the paired sample t test. A p-value of <0.05 was considered statistically significant.

Results and Discussion

All patients completed the study. SOD activity determined at baseline in the groups treated with piroxicam and meloxicam showed no significant differences when compared with each other. In tenoxicam treated group, initial SOD activity was slightly higher than in the other two groups (Table I). The variations of SOD activity for each patient at baseline and at the end of treatment with oxicams are presented in Figure 1.

In patients treated with piroxicam and meloxicam an increase of SOD activity was observed, whereas in patients treated with tenoxicam has been a slight decrease of SOD activity (Figure 2).

Results showed that SOD activity increased significantly only in piroxicam treated group ($p=0.04$) and had no significant variations in tenoxicam and meloxicam treated groups ($p=0.85$ and $p=0.11$, respectively) (Table I).

Table I
Superoxid dismutase (SOD) activity in the groups of study

Oxicam-treated group	Patients' age (mean $\pm$ SD)	Baseline SOD activity (U/ml) (mean $\pm$ SD)	Final SOD activity (U/ml) (mean $\pm$ SD)	p value
Piroxicam	52.8 $\pm$ 4.23	150.15 $\pm$ 25.79	172.68 $\pm$ 20.24	0.04
Tenoxicam	54.2 $\pm$ 2.15	169.05 $\pm$ 33.19	167.12 $\pm$ 19.92	0.85
Meloxicam	61 $\pm$ 10.02	153.15 $\pm$ 21.82	166.22 $\pm$ 27.75	0.11

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

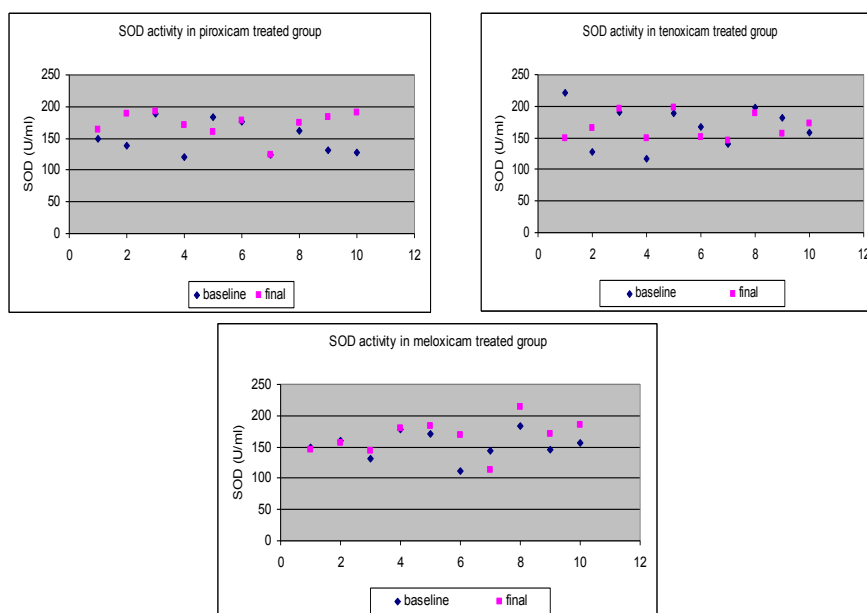


Figure 1

Variation of superoxide dismutase (SOD) activity for each patient from the three groups treated with oxicams

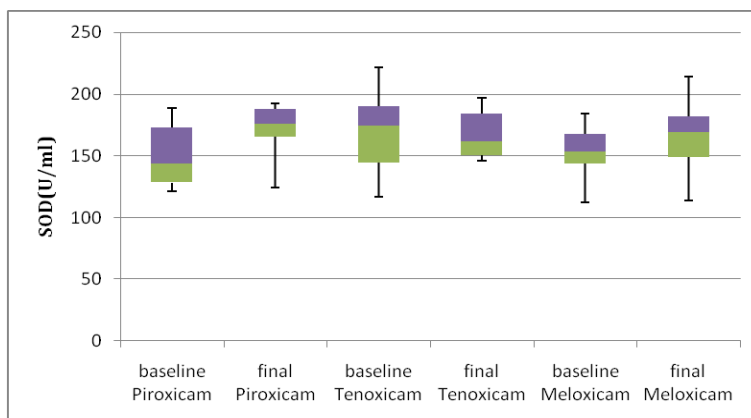


Figure 2

Variation of SOD activity in all the groups treated with oxicams
($p < 0.05$ for piroxicam treated group)

ROS scavenging activity of tenoxicam was tested *in vitro* in many types of non-cellular systems. Tenoxicam was proved to be a good scavenger of hydroxyl and superoxide radicals *in vitro* [4]. Ozgocmen et al. have determined that tenoxicam could have antioxidant effects, and tenoxicam and celecoxib may decrease serum nitrite [7]. Van Antwerpen and Neve studied ROS scavenging activity of piroxicam, tenoxicam, lornoxicam, meloxicam, nimesulide and ibuprofen *in vitro* and found that piroxicam has a higher antioxidant activity than tenoxicam, ibuprofen and nimesulide [14]. It is known that oxidative stress can induce cell death by apoptosis and SOD plays an important role in preventing apoptosis [5].

Regan et al. reported a significant decrease in SOD activity in patients with osteoarthritis [10].

Cimen et al. investigated the *in vivo* effect of celecoxib, ibuprofen and tenoxicam on free radicals metabolism in erythrocytes from patients with osteoarthritis and found a similar influence on SOD and antioxidant potential of these NSAIDs, despite the different mechanism of COX inhibition [2].

Tuzun et al. reported that tiaprofenic acid and flurbiprofen significantly increase SOD activity in plasma [13], while Ozgocmen et al. showed that tenoxicam and celecoxib causes an insignificant increase of SOD activity in patients with osteoarthritis [7].

Our findings – a significant influence of piroxicam (a nonselective COX inhibitor) on SOD activity, but an insignificant effect of tenoxicam (also a nonselective COX inhibitor) and of meloxicam (a selective COX-2

inhibitor) – are contradictory, suggesting that the effect on SOD activity is not based on COX inhibition.

Conclusions

Increased SOD activity was statistically significant in piroxicam treated group, while tenoxicam and meloxicam did not alter significantly the activity of this enzyme.

Although during prostaglandin synthesis from arachidonic acid under the COX action are generated free radicals, COX inhibition alone does not seem to fully explain the effects demonstrated by oxicams in this study, supporting the hypothesis of an additional mechanism on SOD activity, independent of COX inhibition. Thus, further studies are required to assess the effect of oxicams on oxidative stress in OA patients, which could provide new therapeutic approaches for OA treatment.

References

1. Al-Waili NS, Saloom KY, Al-Waili T, Al-Waili A, Al-Waili H., Modulation of prostaglandin activity, part 1: Prostaglandin inhibition in the management of nonrheumatologic diseases: Immunologic and hematologic aspects, *Adv. Ther.*, 2007; 24(1): 189-222.
2. Cimen MYB, Bolgen-Cimen O, Escandari G, Sahin G, Erdogan C, Atik U., In vivo effect of meloxicam, celecoxib and ibuprofen on free radical metabolism in human erythrocytes, *Drug. Chem. Toxicol.*, 2003; 26: 169-176.
3. Ciurea P, Roşu A, Muşetescu AE, Vreju FA, Reumatologie, Ed. Medicală Universitară, Craiova, 2007; 387-413.
4. Facino RM, Carini M, Saibene L., Scavenging of free radicals by tenoxicam: a participating mechanism in the antirheumatic/antiinflammatory efficacy of the drug, *Arch. Pharm.*, 1996; 329(10): 457-463.
5. Kalaci A, Yilmaz RH, Aslan B, Söğüt S, Yanat AN, Uz E., Effects of hyaluronan on nitric oxide levels and superoxide dismutase activities in synovial fluid in knee osteoarthritis, *Clin. Rheumatol.*, 2007; 26: 1306-1311.
6. Maneesh M, Jayalekshmi H, Suma T, Chatterjee S, Chakrabarti A, Singh TA., Evidence for oxidative stress in osteoarthritis, *Indian J. Clin. Biochem.*, 2005; 20(1): 129-130.
7. Ozgocmen S, Ardicoglu O, Erdogan H, Fadillioglu E, Gudul H., In vivo effect of celecoxib and tenoxicam on oxidant/anti-oxidant status of patients with knee osteoarthritis, *Ann. Clin. Lab. Sci.*, 2005; 35(2): 137-143.
8. Perry JJP, Shin DS, Getzoff ED, Tainer JA., The structural biochemistry of the superoxide dismutase, *Biochim. Biophys. Acta*, 2010; 1804: 245-262.
9. Rees MD, Kennett EC, Whitelock JM, Davies MJ., Oxidative damage to extracellular matrix and its role in human pathologies, *Free Radical Biol. Med.*, 2008; 44: 1973-2001.
10. Regan E, Flannelly J, Bowler R. et al., Extracellular superoxide dismutase and oxidant damage in osteoarthritis, *Arthritis Rheum.*, 2005; 52: 3479-3491.
11. Sarhan S, Kocyigit A, Yazar M, Isikan UE., Plasma total antioxidant capacity, lipid peroxidation, and erythrocyte antioxidant enzyme activities in patients with rheumatoid arthritis and osteoarthritis, *Clin. Biochem.*, 2005; 38: 981-986.
12. Porfire A.S., Tomuța I., Leucuța S.E., Achim M., Superoxide dismutase loaded liposomes. The influence of formulation factors on enzyme encapsulation and release. *Farmacia*, 2013; 61(5): 865-873.

13. Tuzun S, Uzun H, Aydin S, Dinc A, Sipahi S, Topcuoglu MA, Yucel, R; Belce A., Effects of flurbiprofen and tiaprofenic acid on oxidative stress markers in osteoarthritis: A prospective, randomized, open-label, active- and placebo-controlled trial, *Curr. Ther. Res.*, 2005; 66(4): 335-344.
14. Van Antwerpen P, Nève J., In vitro comparative assessment of the scavenging activity against three reactive oxygen species of non-steroidal anti-inflammatory drugs from the oxicam and sulfoanilide families, *Eur. J. Pharmacol.*, 2004; 496: 55-61.
15. Zhang W, Moskowitz RW, Nuki G, Abramson S, Altman RD, et al., OARSI recommendations for the management of hip and knee osteoarthritis, Part II: OARSI evidence-based, expert consensus guidelines, *Osteoarthr. Cartilage*, 2008; 16: 137-162.
16. Ziskoven C, Jäger M, Zilkens C, Bloch W, Brixius K, Krauspe R., Oxidative stress in secondary osteoarthritis: from cartilage destruction to clinical presentation?, *Orthop. Rev.*, 2010; 2: e23.

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