JOTCS A
Journal of the Turkish Chemical Society
Section: A
Chemistry
TURKISH CHEMICAL SOCIETY

Biochemical Confirmation of Anti-Inflammatory Activity of Oxicam-Based Pharmaceutical Compositions

Syrova Ganna Olegivna, Tishakova Tetyana Stanislavivna*, Levashova Olga Leonidivna, Savelieva Olena Valeryivna

*Kharkiv National Medical University, Medical and Bioorganic Chemistry Department, 61022, Kharkiv, Ukraine.

Abstract: Biochemical confirmation of anti-inflammatory activity of oxicam-based pharmaceutical compositions was performed by the determination of the level of one of the main markers of inflammation-C-reactive protein. Biochemical studies were carried out on laboratory animals (white WAG rats) to study the anti-inflammatory effects of meloxicam, piroxicam, caffeine, and pharmaceutical compositions consisting of meloxicam and caffeine, piroxicam and caffeine compared to the reference drug - sodium diclofenac. The content of CRP in serum of rats was determined using the CRP latex test kit. It was shown that the composition of meloxicam and caffeine reduced the content of CRP by 16 times compared with formalin-induced edema, and by 2 times in comparison with the reference drug diclofenac sodium, which is statistically significantly different from the control.

Keywords: Anti-inflammatory activity, pharmaceutical composition, C-reactive protein.

Submitted: September 25, 2018. **Accepted:** December 26, 2018.

Cite this: Syrova G, Tishakova T, Levashova O, Savelieva O. Biochemical Confirmation of Anti-Inflammatory Activity of Oxicam-Based Pharmaceutical Compositions. JOTCSA. 2018;5(3):1407–12.

DOI: http://dx.doi.org/10.18596/jotcsa.463663.

***Corresponding author.** E-mail: ttishakova@ukr.net. Tel: (+380502982104).

INTRODUCTION

Modern medicine has a wide arsenal of anti-inflammatory drugs (AID), because an inflammatory process is a leading pathogenetic link of many diseases, including rheumatic diseases and musculoskeletal system diseases, which constitute about 80% of the pathology in any medical practice. However, along with pharmacological action and sufficient degree of clinical efficacy, most of them cause a number of undesirable side effects (1, 2).

The search of highly effective pharmacological compositions with minimal side effects is a relevant issue. One of these areas is the creation of pharmacological compositions based on nonsteroidal anti-inflammatory drugs (NSAIDs).

Considering the fact that both cyclooxygenase isoenzymes participate in the pathogenic mechanisms of the pain syndrome (acute pain syndrome) development, application of nonselective drugs with a balanced inhibitory activity with regard to cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2) is the most viable. Piroxicam suppresses the production of prostaglandins in the area of inflammation and inhibits the production of "physiological" prostaglandins. It reduces the formation of rheumatoid factor - a protein of the acute phase of inflammation, which belongs to the group IgM; increases the ratio of Th/Ts, therefore, it is able to suppress autoimmune reactions in the area of the inflammatory organ or tissue (5-7).

Selective inhibitors include meloxicam, which differs from other drugs in terms of its high efficacy and safety. Administration of meloxicam decreases the synthesis of prostaglandins and the degree of formation of oxygen-free radicals. Meloxicam readily penetrates into the synovial fluid, which indicates that the active substance contributes to the elimination of the infectious process in the joint tissues. It is 20-fold COX-2 selective as compared to COX-1, meloxicam positively affects the metabolism of cartilage tissue and is characterized by chondroprotective properties (3, 4).

In the proposed pharmaceutical composition, we introduced an adjuvant of NSAIDs and nonnarcotic analgesic (NNA) - caffeine (8, 9-11). Furthermore, the enhancement of the analgesic effect of NNA is associated with the central cholinergic analgesic effect of caffeine (12), with the structural similarity of adenosine and caffeine molecules that contributes to the neurochemical mechanism of its action in blocking specific P1-purine receptors in the brain (13).

C-reactive protein analysis (CRP) is a non-specific indicator of inflammation. The amount of protein significantly increases in the presence of inflammatory process of any etiology, including tumoral and necrotic processes. That is why CRP is considered as a non-specific inflammatory marker. CRP enhances the mobility of leukocytes. By binding to T-lymphocytes, it affects their functional activity initiating precipitation, agglutination, phagocytosis, and complement fixation. CRP level in the blood elevates in 4 hours after onset of inflammation and disappears during the convalescence. In the presence of calcium, CRP binds ligands in polysaccharides of microorganisms and causes their elimination. The level of CRP in the serum shows the intensity of the inflammatory process. Control of CRP is an important for monitoring different inflammatory diseases (14, 15).

Our quantum chemical investigations have shown that the chosen reference drug - diclofenac is the mildest reagent in comparison with piroxicam and meloxicam. The absolute hardness of diclofenac is 2.8746, and for piroxicam and meloxicam, 4.0569 and 4.1189 respectively (16). It is also known that meloxicam is a selective COX-2 inhibitor and diclofenac with piroxicam are nonselective COX-1 and COX-2 inhibitors.

MATERIALS AND METHODS

Biochemical studies were carried out on laboratory animals (white WAG rats) in order to study the anti-inflammatory effects of meloxicam, piroxicam, caffeine, and pharmaceutical compositions consisting of meloxicam and caffeine, piroxicam and caffeine compared to the reference drug - sodium diclofenac.

The anti-inflammatory action of the above mentioned substances was studied by the experimental model of formalin-induced paw edema.

The rats were divided into 8 groups of 6 animals each. Animals of the 1st intact group intragastrically received single dose of 3% starch mucus (2 mL per 200 g of rat's weight). Animals of the 2nd group received 3% starch mucus and the formalin induced edema was modeled by sub-planar administration of 2% formalin solution into hind paw of rat. Animals from experimental groups 3-8 were intragastrically administered studied compositions in the form of a suspension of 3% starch mucus. Animals of the 3rd group - piroxicam in the dose of 1.3 mg per 1 kg of bodily weight, 4th -meloxicam in a dose of 0.6 mg per 1 kg of bodily weight, 5th - caffeine (0.6 mg per 1 kg of rat's weight), 6th group received composition of piroxicam (1.3 mg per 1 kg of rat's weight) with caffeine (0.6 mg per 1 kg of rat's weight), 7th group - composition of meloxicam (0.6 mg per 1 kg of rat's weight) with caffeine (0.6 mg per 1 kg of rat's weight), 8th group – reference medicinal product (8 mg of sodium diclofenac per 1 kg of rat's weight). Maximum development of formalin induced edema is observed 4 hours after its modeling. 3% starch mucus, drugs and their pharmaceutical compositions were administered 1 hour before, taking into account their pharmacokinetic and pharmacodynamic characteristics. Animals of all groups were decapitated under etheric anesthesia (17). Blood collection was carried out in rats of all groups. After the blood collection of blood samples of all groups of animals had been subjected to centrifugation at 1500 rpm/min for 15 minutes. The whole plasma was collected and subjected to CRP analysis using standard latex test.

The content of CRP in serum of rats was determined using the CRP latex test kit (State registration number 1248/2002, Kharkiv, Ukraine). The method is based on the detection of acute phase protein in the serum – CRP, which enters agglutination reaction with anti-CRP antibodies, adsorbed on neutral latex particles. Agglutination of latex particles is considered a positive reaction, indicating the presence of C-reactive protein at a significant and detectable level. Specimens which do not contain human CRP will not cause agglutination.

RESULTS AND DISCUSSION

The level of CRP in serum of intact rats was 6±0.004 mg/L. This indicator increased significantly and reached value 96±0.001 mg/L under the condition of formalin induced edema, which exceeds the norm by 16 times.

The biochemical study of the anti-inflammatory activity of piroxicam, meloxicam, caffeine and their composition on the content of the inflammation marker CRP showed that the investigated drugs had an effect on the CRP content in rat's serum and significantly lowered it regarding the formalin induced edema (**Table 1**).

Decrease in CRP level in the serum of rats 2 times as compared with formalin edema was observed after mono-administration of non-selective COX-1 inhibitor (piroxicam), but the obtained data statistically significant differ from the reference product diclofenac sodium: the effect of piroxicam is 4 times less than the reference medicinal product for the content of CRP in the serum of rats.

Mono-administration of the selective COX-2 inhibitor – meloxicam showed a significant decrease of the CRP content in the blood serum of rats under condition of formalin edema. The content of CRP decreased by 8 times and did not statistically significant differ from the diclofenac sodium. Thus, the selective COX-2 inhibitor (meloxicam) more effectively affects the CRP content of serum in rats under conditions of formalin edema than the non-selective COX-1 inhibitor (piroxicam).

The mono-administration of analgesic adjuvant caffeine also reduced the CRP content of rat's serum (24±0.001 mg/L) by 4 times as compared to formalin edema, but it is 2 times less than diclofenac sodium influenced the content of CRP in serum blood in rats.

Composition of piroxicam with adjuvant caffeine reduced the content of the CRP in blood serum of rats by 4 times compared with formalin-induced edema but the obtained data did not reach the reference medicinal product (sodium diclofenac). The pharmaceutical composition of piroxicam with caffeine had 2 times less effect on the content of CRP in the serum of blood in rats then reference drug. Therefore, caffeine potentiates the anti-inflammatory effect of piroxicam in this composition.
The composition of meloxicam and caffeine proved to be the most effective and worked better than all the investigated drugs, including the reference drug. This composition reduced the content of CRP by 16 times compared with formalin-induced edema, and by 2 times in comparison with the diclofenac sodium, which is statistically significantly different from the control, *i.e.* caffeine increases and potentiates anti-inflammatory action of meloxicam.

Table 1: Anti-inflammatory action of piroxicam, meloxicam, caffeine, and their pharmaceutical compositions on the CRP level in blood serum of rats under conditions of formalin-induced edema (n = 6).

№	Groups of rats	CRP, mg/L
1	Control	6 ± 0.004
2	Formalin-induced edema	96 ± 0.001[1]
3	Piroxicam	48 ± 0.003[1/2/4/5/6/7/8]
4	Meloxicam	12 ± 0.005[1/2/3/5/6/7]
5	Caffeine	24 ± 0.001[1/2/3/4/7/8]
6	Piroxicam + caffeine	24 ± 0.002[1/2/3/4/7/8]
7	Meloxicam + caffeine	6 ± 0.001[3/3/4/5/6/8]
8	Sodium diclofenac	12 ± 0.001[1/2/3/5/6/8]

Note 1. (mean ± error in mean)[1] - the difference is significant as compared to the control group, P <0.05;
Note 2. (mean ± error in mean)[2] - the difference is significant as compared to formalin-induced edema, P <0.05;
Note 3. (mean ± error in mean)[3] - the difference is significant as compared to the mono-administration of piroxicam, P <0.05;
Note 4. (mean ± error in mean)[4] - difference is significant as compared to mono-administration of meloxicam, P <0.05;
Note 5. (mean ± error in mean)[5] - the difference is significant as compared to the mono-administration of caffeine, P <0,05;
Note 6. (mean ± error in mean)[6] - the difference is significant as compared to the administration of the piroxicam and caffeine composition, P <0.05;
Note 7. (mean ± error in mean)[7] - the difference is significant as compared to the introduction of meloxicam and caffeine, P <0.05;

Note 8. (mean ± error in mean)[8] - the difference is significant as compared to the mono-administration of diclofenac sodium, P <0.05.

Above mentioned results can be represented graphically that helps with visualization of data (Fig. 1):

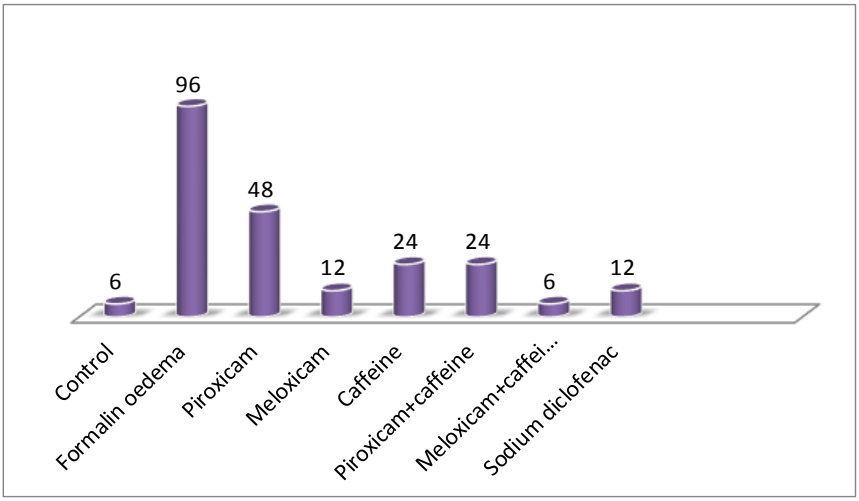

Figure 1. Content of the CRP in blood serum of rats (in mg/L).

CONCLUSION

The results of biochemical studies of anti-inflammatory activity indicate that piroxicam, meloxicam, caffeine and their compositions show the pronounced anti-inflammatory effect against formalin-induced edema. The leader in biochemical studies is a two-component composition of meloxicam and caffeine, which reduces the level of the inflammation marker CRP to the level of control group and acts better than reference drug – sodium diclofenac.

ACKNOWLEDGMENTS

Authors are very grateful to the organizing and scientific committees of International Chemistry and Biology Conference'18 (ChemBioCon'18) for possibility to publish manuscript in JOTCSA.

REFERENCES

1. Isakov VA. Gastropathy associated with the use of non-steroidal anti-inflammatory drugs pathogenesis, treatment, prevention. Clinical Pharmacology & Therapeutics . 2005; 14(2): 34—8. [in Russian]

2. Nasonov EL, Nasonova VA. The pharmacotherapy of pain: rheumatologist's glance. Consilium medicum. 2000; 2(12):509-14. [in Russian]

3. Scherbenkov IM. Problems of perioperative anaesthesis. Consilium Medicum. 2011; 8: 78-81. [in Russian]

4. Nasonov EL, Zvetkova ES, Balabanova RM. New aspects of anti-inflammatory therapy of rheumatic diseases: theoretical background and clinical application of meloxicam. Clinical medicine. 2006; 4: 4-8. [in Russian]

5. Nakonechna SA, Honcharenko MM, Aleksieieva TM. Experimental study of antipyretic effect of paracetamol composition with caffeine. Experimental and clinical medicine. 2015; 1(66): 47-9. [in Ukrainian]

6. Dix P, Prosser DP, Streete P. A pharmacokinetic study of piroxicam in children. Anaesthesia. 2004; 59:984-7.

7. Abdallah FI, Dawaba HM, Mansour A *et al*. Evaluation of the anti-inflammatory and analgesic effects of piroxicamloaded microemulsion in topical formulations. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(2):66-70.

8. Boiko Ye. P., Lymanska G.O., Petunin P.O., Bachinskyi R.O. Study of the effect of caffeine composition with ibuprofen on the central component of nociceptive response in rats. Contemporary theoretical and practical aspects of clinical medicine: materials of the international conference of students and young

scientists devoted to the 155th anniversary of V.V. Podvysotsky's birth. Odesa. 2012; 116. [in Ukrainian]

9. Syrova GO, Zvyagintseva TV, Chekman IS *et al*. Quantum-pharmacological substantiation of potentiating anticoagulant properties of caffeine. Pharm. Journal. 2008; 6:85-91. [in Ukrainian]

10. Syrova GO, Zvyagintseva TV. Study of potentiating anticoagulant properties of caffeine in an experiment. Congress of the World Federation of Ukrainian Medical Associations: book of abstracts. Ivano-Frankivsk. 2008; 454. [in Ukrainian]

11. Boiko I, Syrova G, Ermolenko T. Experimental conformation of the caffeine's potentiation of the analgetic properties. 3th International Scientific interdisciplinary Congress of medical students and young doctors. Kharkiv. 2010; 14. [in Russian]

12. Ghelardini C, Galeotti N, Bartolini A. Caffeine induces central cholinergic analgesia. Naunyn Schmiedebergs Arch Pharmacol. 1997; 356(5):590-5.

13. Karelov AE, Zaychik AM, Lebedinskiy KM. Purine analgesia: From Theory to Practical Implementation. Proceedings of the III Congress of pharmacologists of Russia, St. Petersburg. 2007; 7(1): 1718 [in Russian].

14. Mazon SB, Monte AS, Almeida EA, Guariento ME. Determining the C-Reactive Protein Level in Patients With Different Clinical Forms of Chagas Disease. Rev Esp Cardiol. 2010; 63(9):1096–9.

15. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest. 2003; 111(12):1805–12.

16. Syrova GO, Levashova OL, Tishakova TS, Kozub SN, Chalenko NN. Investigation of quantum-chemical properties of indomethacin. The scientific heritage. 2017; 16(16):11-16.

17. Preclinical studies of drugs. Methodological recommendations edited by O.V. Stefanova. Kyiv, 2001; 527. [in Ukrainian]