





Biochemical Confirmation of the Anti-Inflammatory Effect of Coxibs and their Compositions with N-Acetylgulcosaminil 1-4-N-Acetylmuramoil-L-alanyl-D-Isoglagamin

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Abstract: The current work is focused on the biochemical confirmation of the anti-inflammatory effect of coxibs and their compositions with licopid as an adjuvant for NSAIDs in the treatment of inflammation. The current investigation aimed to compare the anti-inflammatory effect of rofecoxib, celecoxib, licopid, and pharmaceutical compositions consisting of rofecoxib and licopid, celecoxib, and licopid. Sodium diclofenac was chosen as the reference drug. The anti-inflammatory effect of the test substances was studied using an experimental model of formalin-induced paw edema. The level of SA was determined using SialoTest (SPC Eco-Service). A biochemical study of the anti-inflammatory effect of rofecoxib, celecoxib, licopid, and their composition on the content of inflammation marker (SA) showed that almost all studied drugs reduced the content of SA in rat's blood serum compared to the negative control. The results of the biochemical study showed that rofecoxib has a pronounced anti-inflammatory effect. It acts almost 1.2 times better than celecoxib, and the leader in our biochemical studies is a two-component pharmaceutical composition of celecoxib + licopid at the level of SA in blood serum under formalin-induced edema.

Keywords: coxibs; biochemical confirmation; anti-inflammatory effect; pharmaceutical composition.

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1. Introduction

Pain management is a major global problem according to the statistics of health services and experts. Nonsteroidal anti-inflammatory drugs (NSAIDs) are common medicines that help relieve pain intensity and reduce inflammation caused by tissue damage. Along with inflammatory diseases, they are used in various pathological conditions characterized by acute and chronic pain, primarily in the musculoskeletal system [1, 2].

NSAIDs are effective in treating pain and inflammation. They have a unique combination of analgesic, anti-inflammatory, and antipyretic effects. According to their chemical structure, they are divided into the following groups: salicylates, oxcams, coxibs, pyrazolidines, and derivatives of indolocytic, phenylacetic, and propionic acids, as well as alkanones and sulfonamide derivatives [3-5].

Chronic pain is accompanied by the activation of cyclooxygenases (COX)-2 and the formation of a large number of pro-inflammatory prostaglandins. Therefore, selective

inhibitors (coxibs) demonstrate efficacy associated with inflammation and analgesia. In addition, they tend to accumulate in the site of inflammation. Coxibs' selectivity is the reason for their use in the treatment of osteoarthritis, rheumatoid arthritis, and acute postoperative pain. Thus, we choose COX-2 selective NSAIDs - coxibs for our study. The most important advantage of coxibs is their selectivity that is 2-3 times higher to COX-2 compare to COX-1. This fact is associated with the development of pathology of the gastrointestinal tract (GIT) and a decrease in the synthesis of "cytoprotective" prostaglandins [6-10].

Celecoxib (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-pyrazol-1-yl]benzenesulfonamide) selectively inhibits COX-2 and blocks the formation of pro-inflammatory PG. Rofecoxib (4-[4-(methylsulfonyl)phenyl]-3-phenyl-2(5H)-furanone) highly specifically inhibits COX-2 and the formation of anti-inflammatory prostaglandins. At therapeutic concentrations, none of the drugs inhibit COX-1 [11-16].

The complex use of NSAIDs and adjuvant drugs could be essential for treating moderate pain according to the recommendations of the World Health Organization (WHO). In previous work, we studied the effect of caffeine on the analgesic, anti-inflammatory effect of NSAIDs. Our study comprises the biochemical confirmation of the possible use of licopid as an adjuvant for NSAIDs in treating inflammation [17]. The immune-stimulating and additive effect of muramyl dipeptide (MDP), which is an integral component of the cell wall peptidoglycan, was established by a group of French researchers led by E. Lederer back in 1974 [18].

Our study selected an analog of muramyl dipeptide - licopid (N-acetylglucosaminyl 1-4-N-acetylmuramyl-L-alanyl-D-isoglutamine) as an adjuvant. As a bacterial immunomodulator, it activates all functions and production of phagocytes as non-specific defense factors (lysozyme, etc.), increases the production of immunoglobulins and pro-inflammatory interleukins, which initiates the immune response, enhancing antimicrobial protection [19].

2. Materials and Methods

The biochemical study of the anti-inflammatory effect was performed on laboratory animals (white WAG-line rats). The current investigation aimed to compare the anti-inflammatory effect of rofecoxib, celecoxib, licopid, and pharmaceutical compositions consisting of rofecoxib and licopid, celecoxib, and licopid. Sodium diclofenac (2-(2-(2,6-dichlorophenylamino)phenyl)acetic acid) was chosen as the reference drug.

The anti-inflammatory effect of the above substances was studied using an experimental model of formalin-induced paw edema. The animals were divided into 8 treatment groups of 6 animals each. Intact animals of the 1st group and negative control (2nd group) received a single intragastrical dose of 3% starch mucus (ml/g). Animals of the 3rd positive control group received reference drug sodium diclofenac (8 mg/kg, orally). Animals in treatment groups 4 – 6 received rofecoxib, celecoxib, and licopid at doses of 1.5, 5, and 0.6 mg/kg correspondingly. Animals of group 7 received the composition of rofecoxib (1.5 mg/kg) with licopid (0.6 mg/kg). Treatment group 8 received composition of celecoxib (5 mg/kg) with licopid (0.6 mg/kg). Formalin solution (2%) was injected subcutaneously in the rat's hind paw (groups 3-8).

Formalin-induced paw edema reaches its maximum in 4 hours after modeling. The administration of 3% starch mucus, reference, and tested drugs was performed 1 hour before maximum swelling taking into account their pharmacokinetic and pharmacodynamic

characteristics. Animals of all groups were subject to ether anesthesia. Rats were kept in vivarium according to the rules of humane treatment of laboratory animals. The studies were conducted in compliance with the principles of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes” [20] and the Decree of the First National Congress on Bioethics [21].

Sialic acids (SA), N-acetyl, and N-glycyl derivatives of neuraminic acid were used as biochemical indicators to study the anti-inflammatory effect of the tested substances [22]. These compounds are considered normal components of all tissues and biological fluids of the human and animal bodies. They are important components of glycoproteins and glycolipids. After cleavage from glycoproteins, free SAs inactivate many bacterial and viral pathogenic agents. Therefore, an increase in sialoglycoprotein content in the blood can manifest a compensatory, protective inflammatory response.

The SA level was determined using SialoTest (SPC Eco-Service). 1 ml of hydrolyzed reagent, 2 ml of distilled water, and 0.6 ml of serum were added to the tubes. The contents of the tubes were thoroughly mixed and placed in a water bath for 5 minutes. After that, they were centrifuged for 6 minutes at 3000 rpm. To the collected supernatant (2 ml) were added 0.4 ml of a color-coagulating reagent, incubated in a boiling water bath for 15 minutes, cooled in cold water, then 2 ml of distilled water was added and stirred. The optical density (OD) was measured against distilled water.

The formula calculated the content of the SA:

$$C_{SA} = \frac{E_{opt} \cdot C_{cal}}{E_{cal}} \text{ mmol/L.}$$

The analysis was performed on a photoelectric colorimeter CPC-3 at a wavelength of 540 nm and a cuvette optical path length of 10 mm. The conversion factor (K) was determined before measuring the samples: the optical density (E_{cal}) of the calibrator was measured on the device against distilled water. The formula calculated the conversion factor: $K = 2/E$, where 2 is the concentration of sialic acids in the calibrator (mmol/L). Statistical processing of the results was performed using the software package Statistics 6.0. The reliability of the obtained results was established using Student’s t-test.

3. Results and Discussion

The level of SA in the blood serum of rats in the negative control reaches 3.508 ± 0.03 mmol/L (group 2), which is almost 1.5 times greater than the intact control (group 1) showed.

A biochemical study of the anti-inflammatory effect of rofecoxib, celecoxib, licopid, and their composition on the content of inflammation marker (SA) showed that almost all studied drugs reduced the content of SA in rat’s blood serum compare to the negative control (groups 3-8), see Table 1.

Table 1. Anti-inflammatory effect of rofecoxib, celecoxib, licopid, and their composition on the content of SA in rats’ blood serum under the condition of formalin-induced paw edema.

№	Treatment groups (n =6)	SA, mmol/L
1.	Intact	2.471±0.06
2.	Negative control	3.508±0.03 ¹
3.	Positive control (sodium diclofenac + formalin edema)	2.68±0.04 ^{2/3/5/7}
4.	Rofecoxib + formalin edema	2.891±0.01 ^{2/4/5/7}
5.	Celecoxib+ formalin edema	3.07±0.02 ^{1/7/8}
6.	Licopid + formalin edema	3.2±0.01 ^{1/7/8}

No	Treatment groups (n =6)	SA, mmol/L
7.	Rofecoxib + licopid + formalin edema	2.79±0.03 ^{2/ 5/7/ 8}
8.	Celecoxib + licopid + formalin edema	2.16±0.11 ^{2/3/4/5/6/8}

Note 1. (mean ± error in mean)¹ – the difference is significant compared to the intact group, P < 0.05;

Note 2. (mean ± error in mean)² – the difference is significant compared to the negative control (formalin-induced paw edema), P < 0.05;

Note 3. (mean ± error in mean)³ – the difference is significant compared to mono-administration of rofecoxib, P < 0.05;

Note 4. (mean ± error in mean)⁴ – the difference is significant compared to mono-administration of celecoxib, P < 0.05;

Note 5. (mean ± error in mean)⁵ – the difference is significant compared to mono-administration of licopid, P < 0.05;

Note 6. (mean ± error in mean)⁶ – the difference is significant compared to rofecoxib + licopid composition, P < 0.05;

Note 7. (mean ± error in mean)⁷ – the difference is significant compared to celecoxib + licopid composition, P < 0.05;

Note 8. (mean ± error in mean)⁸ – the difference is significant compared to the positive control (reference drug), P < 0.05.

Mono-administration of rofecoxib (group 4) comprises 2.891 ± 0.01 mmol/L. The result shows a decrease in the SA content 1.3 times in rats' blood serum compared with the negative control group and approached the positive control.

The results of the celecoxib mono-administration (group 5) comprise 3.07 ± 0.02 mmol/L. It indicates only a tendency to reduce the level of SA in the serum of rats relative to formalin-induced paw edema.

Mono-administration of licopid (group 6) gives a tendency to a decrease in the level of SA in rat serum, relative to formalin-induced paw edema (group 2).

The composition of licopid with rofecoxib (group 7) shows a tendency to decrease the SA content in rat's blood serum compared to mono-administration of rofecoxib but did not reach the reference drug data (gr. 3).

The composition of licopid with celecoxib (group 8) contributed to an effective decrease in the SA content in rat's blood serum under formalin-induced paw edema conditions. This composition shows similar results with the positive control (reference drug, group 3). It was not statistically significantly different from the intact group 1, i.e., licopid effectively potentiates the anti-inflammatory effect of celecoxib.

4. Conclusions

The biochemical study of the coxibs anti-inflammatory effect on the SA level in the rat's serum of rofecoxib and celecoxib indicate that rofecoxib has the pronounced anti-inflammatory effect of the tested drugs. It acts almost 1.2 times better than celecoxib.

The immunostimulant – licopid tends to decrease SA level in rat serum relative to formalin-induced paw edema conditions.

The leader in our biochemical studies is a two-component pharmaceutical composition of celecoxib + licopid at the level of SA in blood serum under formalin-induced edema, which reduces the level of SA (inflammation marker) compare to a level of intact control. It works better than the positive control (reference drug).

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Conflicts of Interest

We know of no conflicts of interest associated with publication.

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