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Dual acting COX/LOX nonsteroidal anti-inflammatory drugs versus traditional COX-2 inhibitors

Podwójne działanie COX/LOX niesteroidowych leków przeciwzapalnych w odniesieniu do tradycyjnych inhibitorów COX-2

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs), inhibiting synthesis of prostaglandins by cyclooxygenase (COX), are some of the most commonly prescribed medications in the treatment of inflammatory states. Two COX isoforms, COX-1 and COX-2 have been identified. It has been suggested that constitutive COX-1 is involved in homoeostatic processes, whereas COX-2 is the isoform that plays a major part in the inflammatory process and the pain associated with it. On the basis of this assumption, selective COX-2 inhibitors were developed; these were intended to have the anti-inflammatory properties of classical NSAIDs but without affecting the integrity of the gastric mucosa. Nevertheless, there is accumulating evidence that COX-1 and COX-2 have overlapping actions and that both isoforms are involved in homoeostasis processes, just as both are modulators of inflammatory reactions [7]. Lipooxygenase (LOX) pathway also plays an important role in inflammation. Leucotriens and lipoxins produced via LOX activity play a role in the damage of gastric mucosa [6]. Compounds that combine COX/LOX inhibition are potential new drugs to treat inflammation. Dual inhibitors, by acting on the two major arachidonic acid metabolic pathways – cyclooxygenase (COX) and lipooxygenase (LOX) – possess a wide range of anti-inflammatory activity. Besides that, dual inhibitors appear to be almost exempt from gastric and cardiovascular toxicity, which is the most troublesome side effect of COX inhibitors [2]. Therefore, the purpose of the research was to compare the action of COX-2 selective inhibitor celecoxib and thiazolidin derivatives possessing dual COX/LOX inhibition on processes of lipoperoxidation and activity of the antioxidant protection system in heart tissue and gastric mucosa.

MATERIAL AND METHODS

The structure of this study and animal experimental procedures were approved by the Ethical Committee of Lviv National Medical University. 40 male albino rats weighing 200–250 g were used.

Animals were divided into 4 groups: 1 – intact animals were used as controls; 2 – COX-2 inhibitor celecoxib was introduced per os for 14 days (10 mg/kg); 3 – {2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrolidin-1-yl}-acetic acid was introduced per os for 14 days (10 mg/kg); 4 – {2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrolidin-1-yl}-benzene-sulfonamide was introduced per os for 14 days (10 mg/kg). Under general anesthesia, rats were sacrificed by decapitation and stomach and heart were excised, and opened longitudinally. For histological investigation, additional samples were fixed in 10% formalin, then embedded in paraffin and sections were prepared and stained with hematoxylin-eosin. After that, tissue samples were homogenized in saline (or phosphate buffer pH 6.0) 1:4, centrifuged at 5000 rpm and supernatant was used for determination of biochemical parameters. Lipid peroxidation level was expressed as MDA concentration in homogenates of heart tissue and gastric mucosa. It was measured according to the procedure of Timirbulatow et al. [8]. The content of nitrogen oxide in homogenate was determined by methods of Green, David, 1982 [3]. Activity of enzymes of the antioxidant protection system was evaluated on the basis of determination of SOD [1] and catalase activity by the method of Korolyuk [5].

RESULTS

Under a long-term blockage of COX-2, changes of gastric mucosa morphology in rats were as follows: impaired mucous barrier of gastric mucosa, desquamation of cells, edema. Under the effect of celecoxib, width of gastric mucosa increased by 7% and was 217.96 ± 6.73 μm, the area of transverse section of the nuclei of endocrine cells was 1.82 ± 0.11 μm^2, the area of transverse section of the nuclei of parietal cells was 2.73 ± 0.21μm^2 having increased by 31% (Fig.1). Morphological changes due to injection of {2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrolidin-1-yl}-acetic acid that are evidence of a higher degree of preserved integrity of the mucous barrier components, decreased the number of ulcerative lesions, increased the density of epithelial cells of the surface of mucous membrane, reduced the degree of edema. Due to injection of {2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrolidin-1-yl}-benzene-sulfonamide, the protective effect upon the status of mucous membrane was less manifested whereas in the area of the base of the gastric glands and submucous region morphological changes did not differ from the state of the norm (Fig. 1).

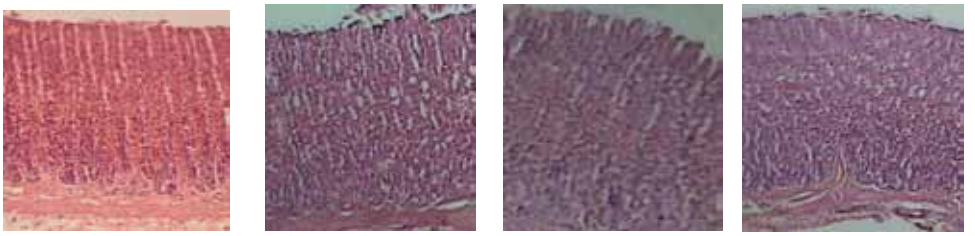

Fig. 1. Histological changes of the gastric mucosa caused by the action of: 2 – celecoxib; 3 –2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-py-rrolidin-1-yl}-acetic acid; 4 – {2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrolidin-1-yl}-benzene-sulfonamide in comparison with – 1 – intact animals gastric mucosa. Magnification of 1:56

COX-2 inhibition by celecoxib caused the increase of MDA content in heart tissue by 37%, indicating activation of lipoperoxidation. After inhibition, both COX and LOX by {2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrolidin-1-yl}-acetic acid and {2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrolidin-1-yl}-benzene-sulfonamide MDA content in heart tissue was also increased, but less than after celecoxib action (by 28% and 30%, subsequently). MDA concentration almost was not changed in gastric mucosa after action of these 3 types of inhibitors (Fig. 2).

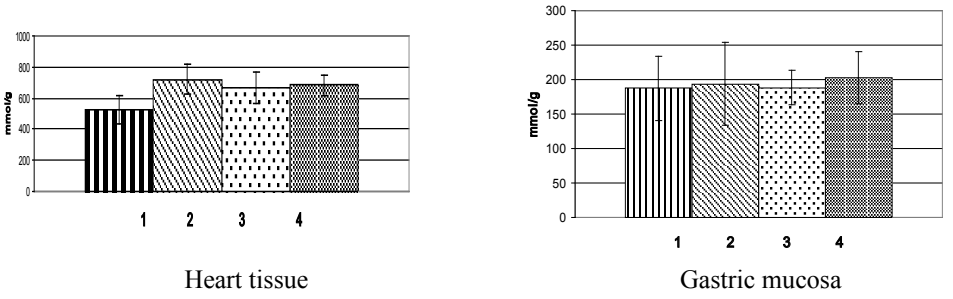

Fig. 2. MDA concentration in heart tissue and gastric mucosa: 1 – control group; 2 – celecixib; 3 – 2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-py-rrolidin-1-yl}-acetic acid; 4 – {2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrolidin-1-yl}-benzene-sulfonamide

NO concentration was 21% higher than normal in heart tissue after COX-2 blockage, whereas agent 2 caused increase of NO concentration only by 7%. NO content almost was not changed in gastric mucosa after celecoxib action. COX/LOX dual inhibition led to a considerable rise in NO concentration in gastric mucosa (by 40% and 22%) (Fig. 3).

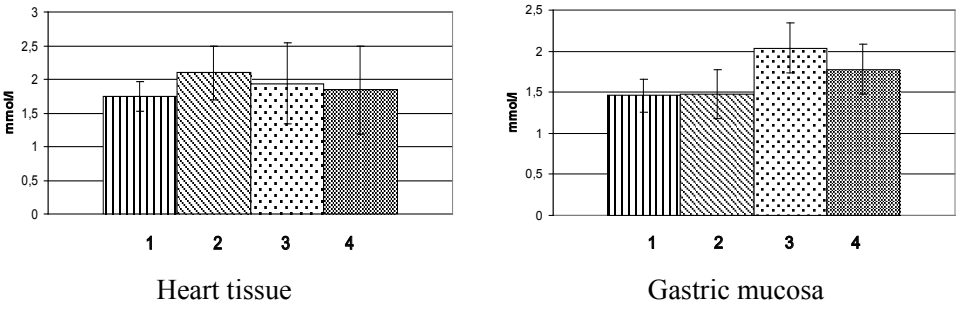

Fig. 3. NO content in heart tissue and gastric mucosa: 1 – control group; 2 – celecixib; 3 – 2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-py-rrolidin-1-yl}-acetic acid; 4 – {2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrolidin-1-yl}-benzene-sulfonamide

Inhibition of COX-2 as well as COX/LOX dual inhibition led to the increase activity of the antioxidant protection system enzymes (catalase, SOD) in both investigated tissues. Celecoxib possesses antioxidant properties itself and increases the activity of SOD (fig. 4). Celecoxib application caused increased catalase activity in heart tissue by 28%, in gastric mucosa by 26%, {2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrolidin-1-yl}-acetic acid increased catalase activity in heart tissue by 24%, in gastric mucosa by 29%, {2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrolidin-1-yl}-benzene-sulfonamide enhanced catalase activity in heart tissue by 20%, in gastric mucosa 23%.

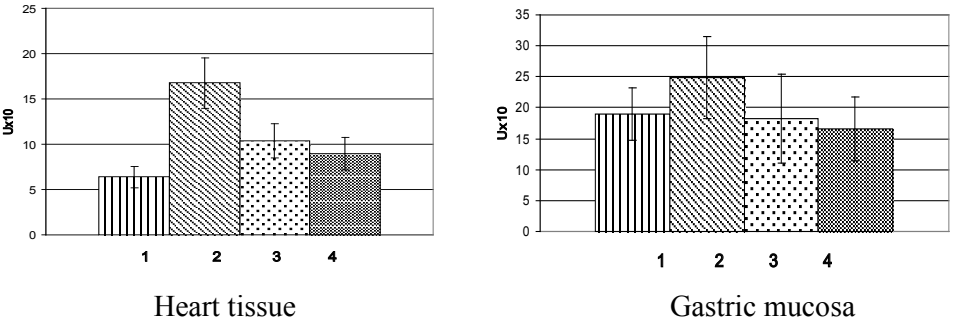

Fig. 4. Activity of SOD in heart tissue and gastric mucosa: 1 – control group; 2 – celecixib; 3 – 2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-py-rrolidin-1-yl}-acetic acid; 4 – {2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrolidin-1-yl}-benzene-sulfonamide

DISCUSSION

Recent investigations have established that COX-2 selective inhibitor celecoxib in addition to its direct anti-inflamatory action can also act through COX-independent mechanisms. Currently, celecoxib derivatives have been developed to inhibit PKB/AKT or to disrupt the mitochondrial membrane potential and have anticarcinogenic activity without inhibiting COX [4]. This indirect action of celecoxib could be a reason why we have obtained morphological changes in gastric mucosa after prolonged application of this inhibitor.

Celecoxib did not cause any significant metabolic changes in gastric mucosa as well as thiazolidin-derivatives, possessing dual COX/LOX inhibition. It was shown before [6] that COX-2 selective inhibitors do not own gastrotoxicity. At the same time, their use is associated with the increase of cardiovascular risk by creating an imbalance between thromboxane A_2 and prostaglandin I_2, leading to vasoconstriction and thrombosis. In our investigations it was established that under prolonged inhibition of COX-2 lipoperoxidation processes in heart tissue where intensified, which can be the result of its thrombotic action.

CONCLUSIONS

Long-term blockage of COX-2 caused the impairement of mucous barrier of gastric mucosa, desquamation of cells, edema. Morphological changes due to injection of dual COX/LOX inhibitors which are evidence of a higher degree of preserved integrity of the mucous barrier components, decreased the number of ulcerative lesions, increased the density of epithelial cells of the surface of mucous membrane, reduced the degree of edema.

COX-2 inhibition by celecoxib led to intensification of lipoperoxidation processes in heart tissue. Activity of enzymes of the antioxidant protection system was increased under these conditions.

Changes in NO content and activity of lipoperoxydation processes after prolonged dual COX/LOX inhibition were less marked in both tissues, comparing with the action of celecoxib.

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SUMMARY

Dual COX/LOX inhibitors, by acting on the two major arachidonic acid metabolic pathways – cyclooxygenase and lipooxygenase, possess a wide range of anti-inflammatory activity. Besides that, dual inhibitors appear to be almost exempt from gastric and cardiovascular toxicity. COX-2 prolonged inhibition by celecoxib led to intensification of lipoperoxidation processes in heart tissue. Activity of enzymes of the antioxidant protection system was increased under the action of celecoxib as well as under the action of thiazolidin-derivatives, possessing dual COX/LOX ingibitory activity. Changes after prolonged dual COX/LOX inhibition were less marked in both tissues, comparing with the action of celecoxib.

Keywords: NSAIDs, cyclooxygenase, lipooxygenase, lipoperoxydation, nitric oxide

STRESZCZENIE

Podwójne inhibitory COX/LOX, poprzez działanie na dwa główne szlaki metaboliczne kwasu arachidonowego – cyklooksygenazę i lipooksygenazę, posiadają szerokie spektrum aktywności przeciwzapalnej. Ponadto podwójne inhibitory wydają się prawie całkowicie pozbawione działań toksycznych na żołądek i układ sercowo-naczyniowy. Długotrwałe hamowanie COX-2 przez celekoksyb prowadzi do intensyfikacji procesów lipoperoksydacji w tkance serca. Aktywność enzymów ochronnego systemu antyoksydacyjnego była zwiększona pod działaniem celekoksybu, podobnie jak w efekcie działania pochodnych tiazolidynowych, posiadających podwójną aktywność hamującą COX/LOX. Zmiany pojawiające się po długotrwałym stosowaniu inhibitorów COX/LOX w obydwu narządach były jednak w mniejszym stopniu zaznaczone niż po stosowaniu celekoksybu.

Słowa kluczowe: NSAID, cyklooksygenaza, lipooksygenaza, lipoperoksydacja, tlenek azotu