

Changes of L-arginine Metabolism in Rat's Colon Mucosa under the Conditions of COX/LOX Inhibition and Acute Stress Action

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L-arginine is a semi-essential amino acid and a precursor of many biologically active compounds. Polyamines and NO produced from L-arginine take part in the regulation of biochemical processes in colon mucosa. Emotional stress, nonsteroidal anti-inflammatory drugs (NSAIDs) and their combined action can change the activity of L-arginine metabolizing enzymes. The aim of this study was to investigate the single action of NSAIDs with different mechanisms of action and their combination with acute stress on L-arginine metabolism in colon mucosa of rats. Animals were divided into 8 groups: control group (1), administration of nonselective, COX-2 selective and dual COX-2/5-LOX inhibitors (groups 2-4), acute stress group (5), administration of same NSAIDs as in groups 2-4 under the conditions of acute stress (groups 6-8). The activity of iNOS, cNOS, arginase, concentration of L-arginine, nitrite and nitrate was measured in colon mucosa. Nonselective COX inhibition by naproxen caused the increase in iNOS and decrease in cNOS activity in colon mucosa. Both COX-2 (celecoxib) and dual COX-2/5-LOX (2A5DHT) inhibitors enhanced cNOS and arginase acting in combination with acute stress. The concentration of L-arginine remained unchanged in most of the groups, but combination of dual COX-2/5-LOX inhibitor and acute stress raised this parameter.

Keywords: L-arginine, NO-synthase, arginase, COX/LOX-inhibitors, colon mucosa, stress.

L-arginine and its metabolites are important regulators of biochemical processes in colon mucosa (CM) and whole gastro-intestinal (GI) tract. The turnover of L-arginine in CM takes place in properly colonocytes¹, it regulates the proliferation of intestinal stem cells². Exertive metabolism of L-arginine was also identified in macrophages present in lamina propria³ and in CM biofilm, where it is metabolized by bacteria¹.

Intracellular pool of L-arginine is formed by few factors – protein degradation⁴, transport from intracellular space by amino acid and L-arginine-containing peptides transporters⁵ and endogenous synthesis via intestinal-renal axis⁶. Cationic amino acids transporters (CAT) – CAT-1 and CAT-2 are responsible for L-arginine and other cationic amino acids transport⁷. Endogenous synthesis of L-arginine by intestinal-renal axis covers few

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steps: L-citrulline is synthesized from glutamine, glutamate and proline in the mitochondria of enterocytes, released from the small intestine and taken up by kidneys for L-arginine production via argininosuccinate synthase and argininosuccinate lyase⁸.

There are four main pathways of L-arginine metabolism – oxidative NO-synthase/NO pathway and non-oxidative arginase/L-ornithine, arginine decarboxylase/agmatine and arginine:glycine amidinotransferase/creatine pathways⁹ (Fig. 1). More than 1% of metabolized L-arginine is utilized for polyamine synthesis and near 2% for constitutive NO production in mammalian cells⁶. All the rest L-arginine can be included into protein biosynthesis⁶. L-ornithine can be used by ornithine transaminase to provide L-proline¹⁰, which takes part in collagen and other proteins biosynthesis. Arginine:glycine amidinotransferase takes part in creatine synthesis mainly in kidneys, but is also present in intestines¹¹ (Fig. 1).

NO-synthase (NOS) and arginase are the most active L-arginine metabolizing enzymes of CM¹². There are three isoforms of NOS – neuronal (NOS-1), inducible (NOS-2, iNOS) and endothelial (NOS-3). NOS-1 and NOS-3 are constitutive (cNOS) isoforms; all of them are detected in CM¹³. Both isoforms of arginase – arginase-1 (cytoplasmic isoform) and arginase-2 (mitochondrial isoform) are also found in CM¹⁴. The utilization of L-arginine by NOS/NO pathway in CM is highly augmented in state of stress¹⁵ and inflammation¹⁶ due to infiltration of CM with iNOS-expressing macrophages¹⁷. Polyamines like putrescine, spermidine and spermine protect CM from endotoxins and activate protein synthesis¹⁸, induce the secretion of immunoglobulin A, maintain barrier integrity¹⁹, DNA stabilization, activate ion channel transport, scavenge free radicals and induce cell proliferation²⁰. However, polyamines synthesis can be disturbed under the conditions of elevated consumption of L-arginine by iNOS¹.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are still one of the most wide-used medications²¹. They act by inhibition of cyclooxygenase (COX) pathway of arachidonic acid (AA) metabolism. It results in downregulation of prostaglandins (PGs), mainly prostaglandin E₂ (PG E₂) synthesis²². Basal level of PG E₂ synthesis

is carried out by constitutive isoform of COX (COX-1) in epitheliocytes, located in lower half of crypts²³. PG E₂ binds with PGE₂ receptors and activates the production of mucus by CM via cAMP dependent mechanism²⁴. Thromboxane A₂ (TX A₂) and prostacyclin (PG I₂) synthesis is also catalyzed by COX-1, they ate blood flow and thrombocytes aggregation²⁵. Expression of inducible COX-2 is activated in the apical epitheliocytes of villi and inflammatory cells of lamina propria in case of inflammation²⁶. The large intestine shows relatively low levels of COX-1 expression compared with that of other parts in GI tract²⁷. Sharply increased synthesis of PG E₂ by COX-2 can determine further inflammation reactions after binding with PGE₂ receptors located on mast cells, T-helpers²⁸ and other immune cells. Thus, inhibition of excessive PG E₂ synthesis is the base of anti-inflammatory action of NSAIDs.

However, it is well-known that long-term and/or high-dose administration of NSAIDs can cause ulcers of GI mucosa²⁹. Ulcerogenic action of NSAIDs is connected with mechanism of their action, mainly with the COX selectivity³⁰. Declined activity of COX-1 leads to lower the production of mucus³¹, so CM becomes more sensitive to endotoxins, flagellins and bacterial lipopolysaccharides (LPS) action. In this regard, COX-2 selective NSAIDs are actively synthesized and studied nowadays. However, COX inhibition can cause the shift of AA metabolism on lipoxygenase (LOX) pathway³², which flows out in raised production of proinflammatory leukotriene B₄ (LT B₄) by 5-LOX isoform³³. Dual COX-2/5-LOX inhibitors (darbufelone, licofelone, 2,3-diaryl-xanthenes, chebulagic acid etc.) are considered to be more effective NSAIDs, as they block both pathways of AA metabolism³⁴, while LOX inhibitors can cause the switchover of AA metabolism on COX pathway.

Emotional stress is another, but no less important factor of modern life. Like NSAIDs, stress can cause ulcers in GI mucosa³⁵. The mechanism of its action is associated with adrenaline³⁶, which can cause the vasoconstriction, hypoxia and activation of nitroso-oxidative processes³⁷. On the other hand, slow influence of stress is mediated by activation of hypothalamic-pituitary-adrenal axis via hyperproduction of corticotropin releasing hormone, corticotropin

and cortisol³⁸. Cortisol activates the expression of lipocortin-1, which inhibits phospholipase A₂ – a key enzyme of AA synthesis³⁹. So, both COX and LOX pathways become inactivated like under the action of some NSAIDs.

Taking into account cytoprotective and proinflammatory pathways of L-arginine and AA metabolism, the purpose of this study was to find the cross-links between these pathways. The main parameters of L-arginine metabolism pathways in CM in the normal state, under the conditions of acute stress and action of different COX/LOX inhibitors were defined.

MATERIALS AND METHODS

Animals

All animal procedures were carried out in accordance with international guidelines for the use and care of laboratory animals. The permission was granted by Bioethics Committee of Danylo Halytsky Lviv National Medical University (Protocol '3, 16.03.2015). Total number of 80 12-week-old male outbred albino rats (*Rattus norvegicus domestica*), 200-240 g body weight were obtained from Danylo Halytsky Lviv National Medical University vivarium. All rats were housed in an environmentally controlled room under a 12 h light/dark cycle. There was *ad libitum* access to water and animals were kept on the standard diet in groups of five in cages. All the experiments were performed at the light cycle.

Experimental groups

Animals were randomly divided into 8 groups (Fig. 2), 10 rats in every group. The first group was control, animals were administered NaCl solution and taken out of the experiment after 1 hour. Rats of groups 2-4 were receiving COX/LOX inhibitors by the next scheme: 2nd was administered by nonselective COX inhibitor (naproxen), 3rd – COX-2 selective inhibitor (celecoxib), 4th – dual COX-2/5-LOX inhibitor (2A5DHT) and were taken out of the experiment after 1 hour. The remaining 40 rats were fasting for 24 hours. Water-immersion stress (WIS) was carried out on animals of group 5, after that, they were taken out the experiment. Animals of groups 6-8 were administered the same COX/LOX inhibitors in the same dose and way as rats of groups 2-4. Animals of groups 6-8 were exposed

to the WIS action after 30 min, then they were taken out the experiment. Euthanasia was done by urethane injection.

WIS model

The WIS model was executed as described by K. Takagi⁴⁰, with 5-hours modification⁴¹. Rats were fasting for 24 hours before WIS modeling and had free access to the water. Animals were fixed in plastic boxes in such a way that their body remained stationary. The boxes with rats were immersed into a water bath (t=23 °C), so that their heads were upon the water surface. Animals were kept in this position during 5 hours, water temperature was controlled every 30 min. Euthanasia was performed immediately after the experiment was finished.

Tested compounds, doses and ways of administration

Sterile sodium chloride solution in a concentration 0.9% (Arterium, Lviv, Ukraine) was administered intragastrically in a volume of 1 mL to a control group rats. Three COX/LOX inhibitors were used: nonselective COX-inhibitor naproxen sodium salt (Sigma-Aldrich, St. Louis, MO, USA), selective COX-2 inhibitor celecoxib (American Custom Chemicals Ltd., San Diego, CA, USA), dual COX-2/5-LOX inhibitor 2A5DHT (2-amino-5-(3,5-ditertbutyl-4-hydroxybenzylidene)-thiazol-4-one) – a structural analogue of darbufelone. Compound 2A5DHT (Fig. 3) was synthesized in the laboratory of Department of organic, bioorganic and pharmaceutical chemistry (Danylo Halytsky Lviv National Medical University, Lviv, Ukraine) by method described previously¹⁵. All the tested compounds were administered intragastrically in a single dose of 10 mg/kg, in a volume of 1 mL. Naproxen sodium was dissolved in distilled water, while celecoxib and 2A5DHT were dissolved in water with addition of polysorbate-80 (10% of the total solution volume) for emulsification. Urethane (Sigma-Aldrich, St. Louis, MO, USA) was used for euthanasia in a single dose 4 g/kg, injected intraperitoneally, dissolved in a distilled water.

Tissue homogenates preparation

After euthanasia colons were taken, cut along, washed with 0.9% sodium chloride solution, extra moisture was taken away with filter paper. CM was scraped with glass microscope slides, weighed and put into a test tubes for further homogenization. Cold (0-4 °C) 0.9 % sodium chloride solution was added to CM in a proportion

1:5 (tissue:solution) and CM was homogenized with homogenizer HS-30E (5000 g). Obtained homogenates were additionally centrifuged (1500 g) on cooling (0-4 °C) for 15 min.

NOS assay

NOS activity was evaluated by the activity of L-citrulline production⁴² using the reaction with diacetyl monoxime, antipyrine and iron (II) sulfate diluted in sulfuric acid. Two incubation solutions (pH=7.4) with the same composition, which differed with the presence of Ca²⁺ (for cNOS activity) and sodium ethylenediaminetetraacetate (for iNOS activity) were used. The concentration of L-citrulline was calculated using a calibration curve. Activity of NOS was expressed in imol of L-citrulline/min·mg of protein.

Arginase assay

Total arginase (both arginase-1 and arginase-2) activity was checked by an approach, based on the activity of urea production⁴³. The reaction with á-isonitrosopropiophenone, diluted in ethanol was used for urea detection.

The concentration of urea was revealed using a calibration curve. Activity of arginase was expressed in imol of urea/min·mg of protein.

L-arginine assay

Sakaguchi reaction was used for L-arginine concentration determination⁴⁴. The concentration of L-arginine was found using a calibration curve, it was expressed in imol/g of tissue.

Nitrite-, nitrate-anion assay

The reaction with Griess reagent for nitrite-anion determination and zinc powder to reduce nitrate-anion into nitrite-anion⁴⁵ was used. All the reagents were dissolved in water for injections to avoid the false results, as distilled water may contain nitrite-/nitrate-anion. Absorbance (ë=550) was estimated and nitrite-anion concentration was calculated using a calibration curve. The values in tubes with zinc corresponded to the total concentration of nitrite-/nitrate-anions concentration, which was expressed in imol/g of tissue.

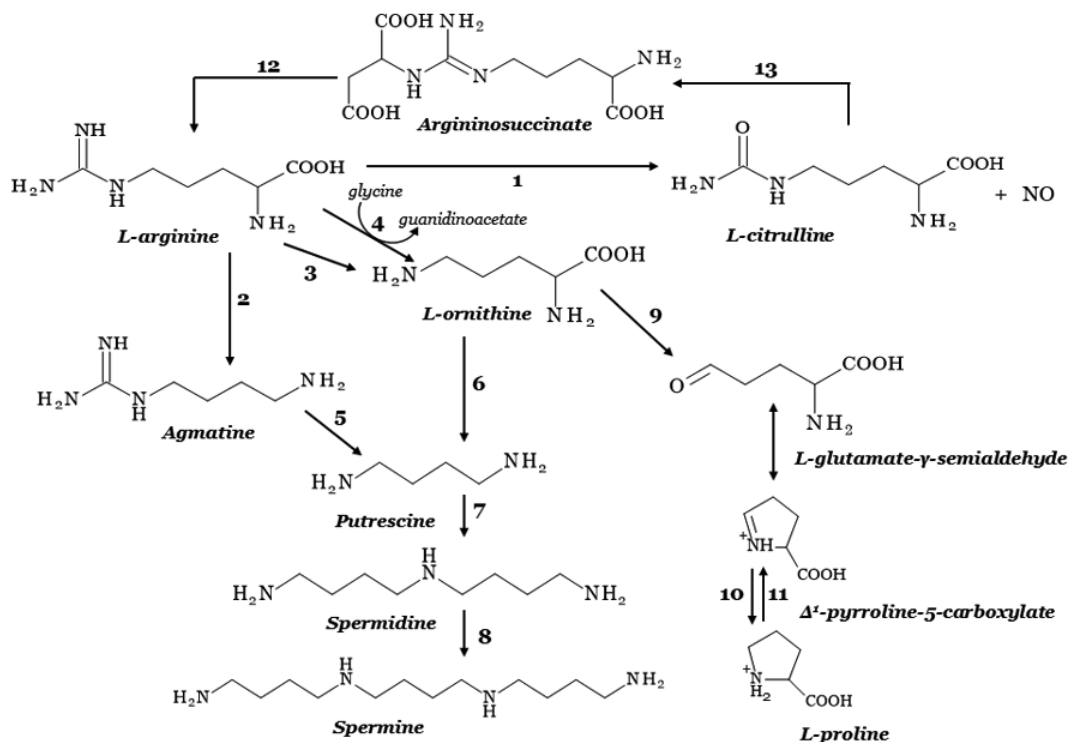


Fig. 1. Pathways of L-arginine metabolism. Notes: 1 – NO-synthase, 2 – arginine decarboxylase, 3 – arginase, 4 – arginine:glycine amidinotransferase, 5 – agmatinase, 6 – ornithine decarboxylase, 7 – spermidine synthase, 8 – spermine synthase, 9 – ornithine aminotransferase, 10 – Δ¹-pyrroline-5-carboxylate reductase, 11 – L-proline oxidase, 12 – argininosuccinate synthase, 13 – argininosuccinate lyase

Protein assay

A modified assay for protein determination was used, which is based on a biuret reaction⁴⁶. The concentration of protein was distinguished using a calibration curve. Concentration of protein was expressed in $\mu\text{mol/L}$.

Statistical analysis

All experimental values are expressed as the mean \pm standard deviation. Descriptive statistics and normality test (Shapiro-Wilk test) were complied, using BioStat Pro Version v6 (BioStat, AnalystSoft Inc., Walnut, CA, USA). The distribution was normal in all the tested groups. Unequal-variance t-test was performed to designate reliability using Excel (Microsoft Office, Microsoft, Redmond, WA, USA). Values $p < 0.05$, $p < 0.01$, $p < 0.001$ were considered statistically significant.

RESULTS

Administration of COX/LOX inhibitors did not change significantly the concentration of L-arginine and nitrite-anion in CM, it affected mainly on NOS and arginase activity.

Thus, nonselective COX inhibitor naproxen administration augmented the iNOS activity by 19% and decreased cNOS activity by 12% ($p < 0.01$) (Fig. 4) in CM compared with that of a control group. Tendency of arginase activity to be lower and nitrate-anion to be higher ($p > 0.05$) was noted.

Celecoxib, like naproxen, did not change the concentration of L-arginine and nitrite-anion. However, it caused iNOS activation by 22% ($p < 0.01$) on the one hand, and tendency of cNOS and arginase activity to be lower ($p > 0.05$) in CM compared to that of a control group (Fig. 4). Like naproxen, celecoxib caused the tendency of nitrate-anion concentration to be elevated ($p > 0.05$).

There were no significant changes of studied parameters in CM under the action of dual COX/LOX inhibitor A25DHT. However, we noted the tendency of arginase activity and nitrite-anion to be lower ($p > 0.05$) compared with that of a control group.

Action of WIS caused the diminishment of L-arginine concentration by 27%, constitutive enzymes' activity – cNOS by 22% and arginase by 24% ($p < 0.001$) in CM compared with that

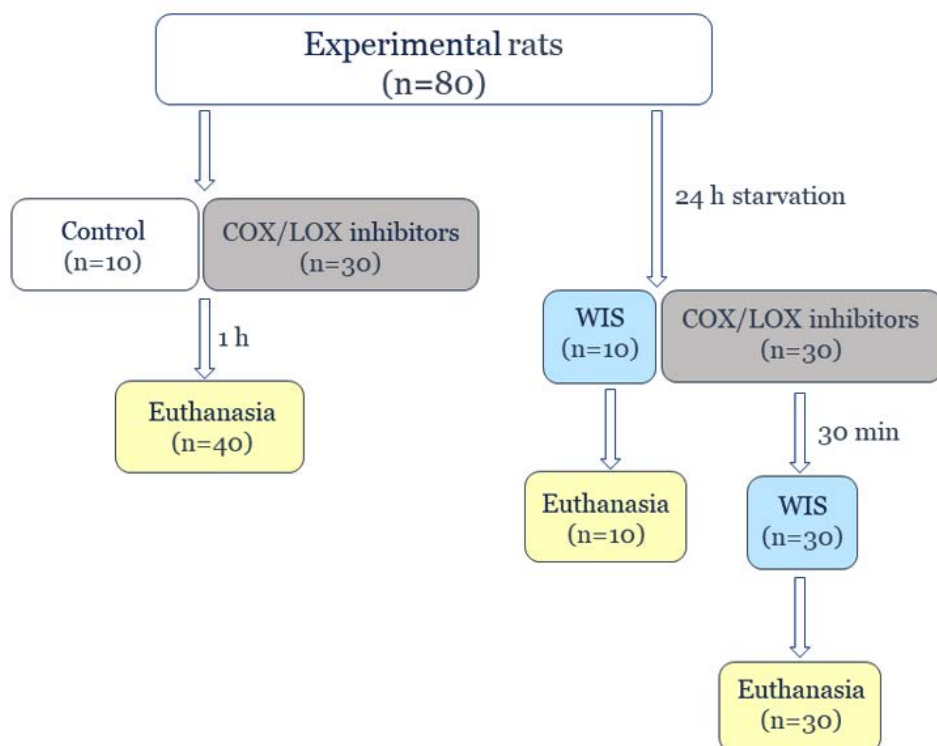


Fig. 2. Design of experiment

of a control group (Fig. 5). At the same time iNOS activity was 2.3 times higher ($p < 0.001$), concentration of nitrite- and nitrate-anion increased by 20% and 27% ($p < 0.05$) compared with that of a control group (Fig. 5).

Administration of COX inhibitors in case of WIS tended to raise L-arginine concentration in CM. Though, the general enzymatic activity did not change significantly compared with that of a WIS group, some interesting changes of enzymes' activity ratio were noted.

Action of naproxen under the conditions of WIS caused the tendency of L-arginine concentration to be elevated ($p > 0.05$), activity of iNOS was lowered by 9% ($p < 0.05$) in CM compared with that of WIS group (Fig. 5). Activity of cNOS, arginase, concentration of nitrite- and nitrate-anion did not change significantly in this case.

The linked action of celecoxib caused the tendency of L-arginine concentration to be augmented ($p > 0.05$), arginase activity was higher

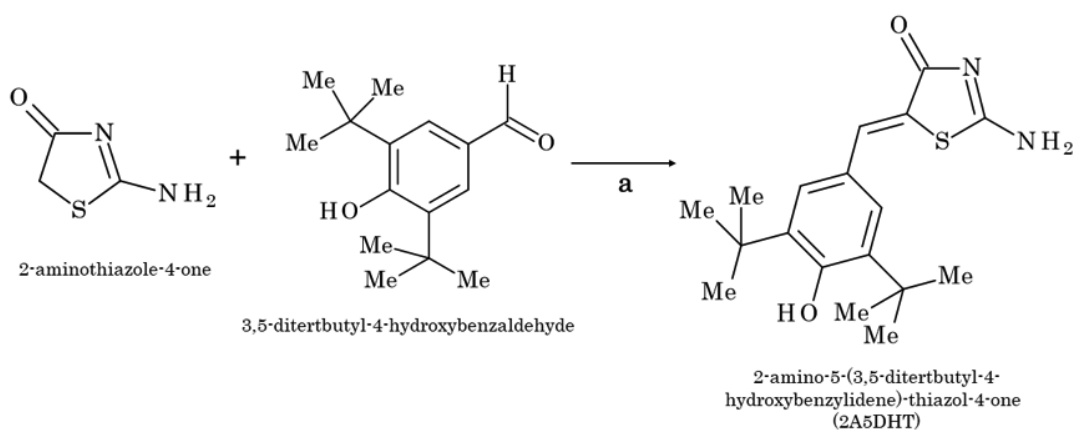


Fig. 3. Synthesis of 2-amino-5-(3,5-ditertbutyl-4-hydroxybenzylidene)-thiazol-4-one (2A5DHT). Reagents, conditions and yields: (a) AcONa, AcOH, reflux 3h, 68%

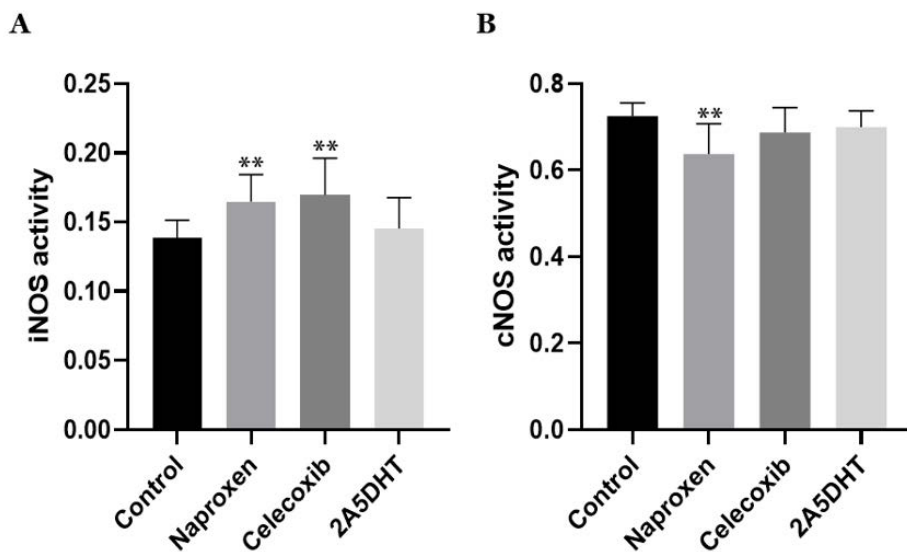


Fig. 4. Activity of enzymes in colon mucosa under the action of COX/LOX inhibitors: A – activity of iNOS, B – activity of cNOS. Notes: ** – $p < 0.01$ compared to the control group

by 12% and iNOS activity – lower by 14% ($p < 0.05$) in CM compared with that of WIS group. Nitrite- and nitrate-anion concentration, cNOS activity remained unchanged (Fig. 5).

Dual COX/LOX inhibitor – 2A5DHT – was the only compound, which increased L-arginine concentration by 17% ($p < 0.05$); iNOS activity was lowered by 15% ($p < 0.01$), while arginase activity was higher by 12% ($p < 0.05$) in CM compared with that of a WIS group (Fig. 5). The tendency of cNOS to be higher ($p > 0.05$) and no changes of nitrite- and nitrate-anion concentration were confirmed.

DISCUSSION

It has to be noted that CM consists of different cell types, where expression of main

L-arginine and AA-metabolizing enzymes is not equable. Moreover, CM is covered by a biofilm, which is inhabited by lots of bacteria capable of metabolizing L-arginine⁴⁷. We consider the inability to study the L-arginine metabolism separately in every cell type and bacterium species as the main limitation of our study.

The first step of this investigation was to find out the influence of a single action of COX/LOX inhibitors on L-arginine metabolism in CM. In general, three possible levels of studied compounds action can be regarded: their action as COX/LOX inhibitors, their topical action as compounds were injected intragastrically and other mechanisms not related to COX/LOX inhibition. All the tested compounds are inhibitors of PG E₂ synthesis, so AA was metabolized by other non-inhibited enzymes. None of studied compounds

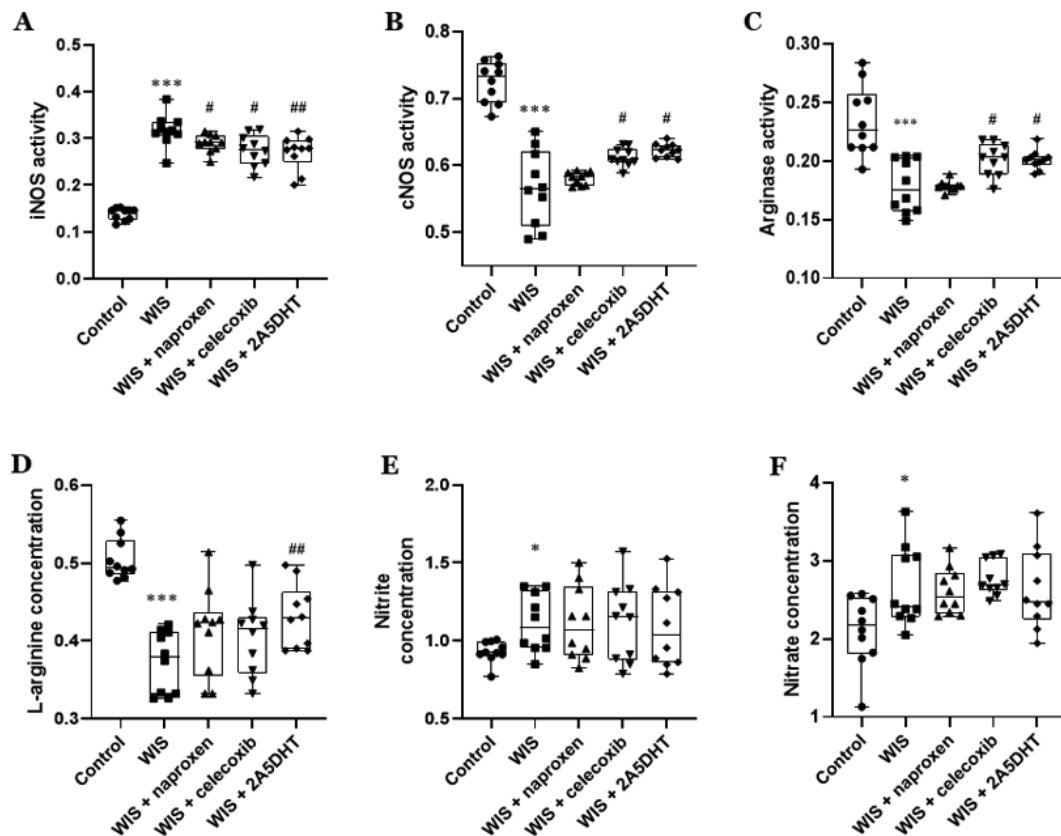


Fig. 5. Combined action of COX/LOX inhibitors and WIS on parameters of L-arginine metabolism in CM: A – activity of iNOS, B – activity of cNOS, C – activity of arginase, D – concentration of L-arginine, E – concentration of nitrite-anion, F – concentration of nitrate-anion. Note. * - $p < 0.05$, *** - $p < 0.001$ compared to control group, # - $p < 0.05$, ## - $p < 0.01$ compared to WIS group

changed the concentration of L-arginine, nitrite- and nitrate-concentration in CM, so our focus was targeted on enzymes activity.

As a result of a nonselective mechanism of naproxen action, it causes a decrease in all the PGs concentration. This results in an elevation of LT B₄ concentration according to excess of AA and ability of LOX, mainly 5-LOX, to metabolize it⁴⁸. PG E₂ is responsible for mucus and bicarbonates secretion in GI mucosa³¹. PG I₂ and TX A₂ are local regulators of the bloodstream⁴⁹. LT B₄ is considered to be a potent proinflammatory agent⁵⁰. The transition of balance between proinflammatory and cytoprotective mediators can cause the activation of inflammation by two mechanisms. The first one is related with macrophages activation by LT B₄ via LTB₄-receptors⁵¹ and later raise in their number in lamina propria⁵². Thus, enhanced activity of iNOS in this group may emerge from macrophages activation⁵³. The second mechanism may be associated with lowered mucus production by cause of PG E₂ deficiency. Mucus protects CM against bacterial LPS, flagellins and toxins supplied by rat's own cells⁵⁴. Moreover, due to the half maximal inhibitory concentration (IC-50) of naproxen, it shows higher affinity to COX-1 (IC-50 35.48 μmol/l) compared with that of COX-2 (IC-50 64.62 μmol/l)⁵⁵. According to our previous investigation, naproxen did not cause histological changes in CM, but the augmentation of free radical oxidation and antioxidant enzymes activity was noted⁵⁶. This fact supports the idea about proinflammatory action of naproxen. The reduction of cNOS and arginase activity may occur by reason of the competition with iNOS for a substrate or as a result of direct action of naproxen on their activity. More expressed changes were obtained in a gastric mucosa – activation of iNOS in gastric mucosa under the action of naproxen led to increased nitrite-anion concentration⁵⁷.

The action of celecoxib – a selective COX-2 inhibitor – was accompanied by an elevation of iNOS activity and tended to decline cNOS activity, which may be caused by competition for L-arginine. As in a case with naproxen, the activity of 5-LOX was not inhibited, the AA was accumulating, and this underlies the raised iNOS activity. However, no changes of arginase activity were noted. It can be explained by not inhibited COX-1 activity, which contributed the

normal production of other PGs. Thus, action of constitutive eicosanoids on arginase activity needs further investigation. Selective COX-2 inhibition demonstrated that the competition between NOS and arginase enzymes is not the only one reason of diversity in inducible and constitutive enzymes' activity. The investigation of celecoxib action on the gastric mucosa showed more expressed changes – enhanced iNOS activity and nitrite-anion concentration, diminished or tended to be diminished cNOS and arginase activity⁵⁷.

Dual COX/LOX inhibitor 2A5DHT caused the tendency of arginase activity to be fallen off, while the activity of NOS isoforms did not change. According to its mechanism of action, 2A5DHT is inhibiting the production of PG E₂ and LT B₄. In this case AA metabolism may be crossed to the local vasoactive PGs production. Both PG I₂ and TX A₂ can be produced, so it remains unclear which one could decrease arginase activity and is this effect related to eicosanoids. We do not connect the lowering of arginase activity with competition for the substrate as the concentration of L-arginine and the activity of NOS isoforms did not change.

By cause of pharmacokinetic properties of tested compounds both naproxen⁵⁸ and celecoxib⁵⁹ are absorbed in 2-4 hours in upper GI tract when administered *per os*. Pharmacokinetic parameters of 2A5DHT are not well-studied. Giving this, we do not support the idea of topical action of naproxen and celecoxib on CM, whereas 2A5DHT theoretically could have this property. Our previous investigation showed that neither celecoxib, nor 2A5DHT cause histological changes or prooxidant action, unlike naproxen⁵⁶. We suppose that the proinflammatory impact of COX/LOX inhibitors can be observed in case of both iNOS activation and cNOS/arginase inhibition, while one of these actions separately does not cause the inflammation.

Action of WIS caused typical changes of studied parameters^{57, 60}. The decline of L-arginine concentration in blood plasma was also noted⁵⁷. We did not measure the concentration of L-arginine in blood plasma as there is a constant flux between the intracellular and extracellular space for L-arginine⁶¹. L-arginine concentration falling off in CM may be caused by a sharp activation of iNOS. Activation of this enzyme is mediated by CM infiltration with macrophages and activation of nonspecific antibacterial action. This process

could be triggered by the action of adrenaline, which causes vasoconstriction⁶². Activation of brain-gut axis cause the inhibition of phospholipase A₂ by cortisol⁶³ and augments visceral sensitivity by corticotropin releasing factor⁶⁴. Glucocorticoids affect the sympathetic nervous system, mediating sensitivity of adrenoreceptors to catecholamines⁶⁵, what may increase their effect. According to the literature data, expression of COX-2 and increase of PG E₂ concentration are raised during the acute stress in rats⁶⁶. Decrease in L-arginine concentration also can be caused by the defect in CAT-2 function in CM in state of inflammation⁶¹. The deficiency of L-arginine is contributing to impairment in epithelial restitution, protein translation, phagocytosis, and innate immunity, as well as dysregulation of apoptosis⁶¹. Defective L-arginine uptake can lead to the diminished ability of arginase/ornithine decarboxylase pathway to generate polyamines⁶¹. Polyamines have many biological functions in CM, including inhibition of Th1 cytokines production, monocyte activation, stimulation of epithelial restitution by enhancing cell migration and proliferation, regulation of apoptosis⁶¹. Literature data are showing controversy affection of glucocorticoids on arginase activity – it can be downregulating⁶⁷ and upregulating⁶⁸. Although it was reported about the co-induction between both arginase-1, arginase-2 and iNOS in macrophages⁶⁸, we noted the opposite action. It can be caused by determination of enzymatic activity in CM homogenates, which consist of different cell types, and macrophages were not the most significant among them. Going back to NOS activity, it can synthesize not only NO, but also N-hydroxyarginine and superoxide-anion⁶⁹. The last one activates arginase, whereas N-hydroxyarginine – inhibits⁶⁹. Sharp elevation of NO production by iNOS in CM caused the elevated concentration of nitrite-/nitrate-anion respectively, what was also noted in gastric⁵⁷ and colon⁷⁰ mucosa. Increased concentration of NO can lead to enhanced formation of peroxynitrite anion, nitration of protein tyrosine, activate nitrosation processes. The last one is regarded to be one of the factors leading to augmented expression of some proinflammatory genes like tumor necrosis factor- α and p38 mitogen-activated protein kinases⁶⁹.

Action of COX/LOX inhibitors in case of WIS action was different compared to their single

action. Naproxen did not show pro-inflammatory impact, moreover the iNOS activity tended to decrease, although it was not enough to shift the L-arginine metabolism on constitutive pathways. We suppose that the reduction of iNOS activity in this case is associated with inhibition of both COX isoforms. In case of a single action, naproxen is supposed to inhibit COX-1 mainly, as the activity of COX-2 is minor. On the other hand, inhibition of COX-1 by naproxen could block the biosynthesis of TXs more than PG I₂, thus increasing the blood flow in the ischemic CM. Related effect of combined action of naproxen and WIS was noted in gastric⁵⁷ and small intestine mucosa⁶⁰.

The coupled action of celecoxib and WIS cannot be considered proinflammatory. However, as described above, the single action of celecoxib enhanced the activity of iNOS. This may point to the cytoprotective role of COX-1 in course of inflammation. On the other hand, WIS action elevates COX-2 activity, which is the main target for celecoxib action. Single administration of celecoxib could partially inhibit COX-1 also, thus causing biochemical changes that led to iNOS activation. Acting on the gastric mucosa under the conditions of WIS, celecoxib declined iNOS activity and did not change cNOS and arginase activity⁵⁷ or diminished arginase activity⁷⁰.

Dual COX/LOX inhibitor 2A5DHT showed the most active regulation of L-arginine-metabolizing enzymes activity in CM in state of WIS action. Uptake of L-arginine and its metabolism by arginase to L-ornithine and conversion to L-proline by ornithine aminotransferase is essential for colonic epithelial lesion repair⁷¹. Moreover, this compound was the only one which increased the concentration of L-arginine in CM. As it was mentioned, WIS may cause ischemia of CM due to augmented adrenaline production. Synthesis of PG I₂, TXs and some other PGs was not inhibited by 2A5DHT. By reason of the reversed vasoactive influence of PG I₂ and TX A₂ and cytoprotective impact of 2A5DHT administration we suppose that AA was metabolized by a prostacyclin synthase mainly.

Interestingly, that the action of all the tested COX/LOX inhibitors did not change the total activity of L-arginine-metabolizing enzymes. Only the administration of dual COX/LOX inhibitor raised the concentration of L-arginine, which can

be used in anabolic pathways. Moreover, according to experimental data, supplementation of rats with L-arginine attenuated the degree of tissue damage in the intestinal ischemia and promoted healing of intestinal mucosa⁶¹. Colonic epithelial restitution is dependent on L-arginine transport into the cells by CAT-2, which is interrupted in case of inflammation⁷¹. According to the literature data, global protein translation is low in the absence of L-arginine⁶. Most studies have demonstrated the beneficial action of L-arginine on GI function improving gastric ulcer healing, accelerating intestinal mucosal regeneration, enhancing bacterial clearance, and reducing histological bowel necrosis⁶. Like NOS isoforms, arginase-1 and CAT-2 are also regarded as inducible, while arginase-2 and CAT-1 – constitutive isoforms⁷². It was reported, that iNOS and argininosuccinate synthetase activities can be co-induced in LPS and interferon- α stimulated macrophages⁷². This underlies the re-synthesis of L-arginine from L-citrulline. We did not observe this hypothetical elevation of L-arginine concentration under the single action of naproxen and WIS, which caused the highest augmentation of iNOS activity. That is why we speculate that L-arginine increasing effect of 2A5DHT may be mediated by PG I₂. It serves for vasodilation, so the concentration of extracellular L-arginine becomes higher. As CAT-2 is an inducible L-arginine transporter localized on macrophages, it is possible that during WIS the transport of L-arginine was increased in all the groups, but catabolized immediately by activated iNOS. The argument for this assumption is lower value of Michaelis constant (Km) of iNOS (13 μ M)⁷³ compared with that of arginase-1 (3.3 mM) and arginase-2 (1.9 mM)⁷⁴.

Our basic assumption is related to the shift of AA metabolism to different directions under the action of COX/LOX inhibitors, which defines proinflammatory or cytoprotective action. An alternative version is associated with accumulation of AA under the action of COX/LOX inhibitors. AA activates calcium entry into the cell, which can stimulate eNOS activity without iNOS inhibition⁶⁹. However, literature data suggest that iNOS and arginase activities are regulated reciprocally in macrophages by cytokines, and this may guarantee the efficient production of NO⁷⁵.

CONCLUSIONS

The single action of nonselective COX inhibitor naproxen can be considered proinflammatory to colon mucosa as it enhances iNOS activity and decreases eNOS/arginase activity. Action of selective COX-2 (celecoxib) and dual COX-2/5-LOX (2A5DHT) inhibitors in combination with acute stress decreased iNOS activity and upregulated eNOS and arginase activity as well. 2A5DHT raised the concentration of L-arginine, showing the most pronounced anti-inflammatory effect. The transition of arachidonic acid metabolism from inducible to constitutive pathways of metabolism can cause a complementary transition of L-arginine metabolism.

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Conflict of interests

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

All animal experiments were done according to Directive 2010/63/EU and approved by Bioethics Committee of Danylo Halytsky Lviv National Medical University (Protocol 13, 16.03.2015).

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