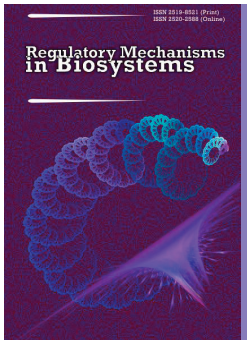

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Arginase/NO-synthase system characteristics in blood lymphocytes under effect of fluoroquinolones

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Antibiotics of the fluoroquinolone series are highly effective synthetic drugs of a wide range of antimicrobial activity. They have a specific mechanism of action on bacterial cells to inhibit the DNA-gyrase enzyme of mostly gram-negative and the topoisomerase IV of gram-positive bacterial cells, which leads to a decrease in the activity of enzymes, disruption of DNA and RNA biosyntheses, and the impossibility of chromosome superspilarization, as a result of which its division is broken and the cell dies. Fluoroquinolones also have an immunomodulatory effect, which is very important in terms of the pathogenesis of many infectious and inflammatory diseases. We assume that fluoroquinolones also act on cells of the body of patients, in particular on such regulatory mechanisms as the arginase-NO-synthase system. In this regard, peripheral blood lymphocytes can be a convenient and adequate model for studying the mechanism of the effect of fluoroquinolones. It is shown that under the influence of various generations of fluoroquinolones, arginase activity increases, depending on the dose, in the following sequence: control → ciprofloxacin → levofloxacin → moxifloxacin. The highest activity is observed under the effects of moxifloxacin, belonging to generation IV. The increase in arginase activity in blood lymphocytes under the influence of fluoroquinolones occurs due to the growth of turnover number of the enzymes (V_{max} increases), although the affinity of enzyme to the substrate decreases (KL-arg increases). At the same time, all fluoroquinolones reduce the activity of the constitutive isoforms of NO-synthase, depending on the dose. It has been established that a slight activity of iNOS of blood lymphocytes in practically healthy women was detected, almost on the verge of error. When studying the influence of fluoroquinolones on the activation of iNOS lymphocytes isolated from the blood of practically healthy women, we did not observe its activity, and the inhibitive effect could not be determined due to its low activity. Oxidative stress was used to induce iNOS activity in blood lymphocytes, with H_2O_2 lymphocytes preincubation. The preincubation of lymphocytes with 0.2 mM H_2O_2 leads to increase of iNOS activity by 31.30 times. By activation of iNOS with hydrogen peroxide, 10^{-5} M concentration of ciprofloxin leads to inhibition of enzyme activity by 1.22 times, levofloxacin by 1.45 and moxifloxacin by 2.34 times. The obtained kinetic parameters suggest that in the blood lymphocytes under the influence of fluoroquinolones, the synthesis of NO with the participation of cNOS is inhibited, and the hyperproduction of NO is inhibited by the activation of iNOS, which is characteristic for pathological conditions.

Keywords: fluoroquinolones; moxifloxacin; levofloxacin; ciprofloxacin; lymphocytes; arginase; NO-synthase.

Introduction

Antibiotics of the fluoroquinolone series are widely used in the system of antimicrobial measures for the prevention and treatment of infectious diseases (Demchuk, 2014; Dudikova et al., 2015; Paliy et al., 2015). These are highly-effective synthetic drugs of a wide range of antimicrobial activity. They have a specific mechanism of action on bacterial cells – to inhibit the DNA-gyrase enzyme of mostly gram-negative and the topoisomerase IV of gram-positive bacterial cells, which leads to a decrease in the enzymes' activity, disruption of DNA and RNA biosynthesis, and the impossibility of hyperspiralization of chromosomes (Boyum, 1968; Almakaeva et al., 2015). The despiralization of DNA of microbial cells occurs, as a result of which its division is broken and the cell dies. This mechanism of action is fundamentally different from antibiotics of other classes, which predetermines the absence of cross-resistance between fluoroquinolones and other antibacterial agents. In addition, these antimicrobial drugs act on the structural elements of the cellular wall of microorganisms, localize the pathogen in the wound, inhibit its penetration into the blood and lymph, inhibit the adhesion of microorganisms to the wound surface and suppress pathogenicity (Kanyuka & Paul, 2011; Majhi et al., 2014; Paliy et al., 2015). The main advantages of fluoroquinolones are their wide range of antimicrobial actions, bactericidal type of action, high antimicrobial activity, in particular regarding bacteria resistant to other antibacterial agents, action on bacteria with intracellular localization, slow adaptation of microorganisms to their action, high bioavailability at peroral application, high intracellular concentration, duration of effects more than 12 hours, low toxicity and high tolerability.

Fluoroquinolones also cause immunomodulatory effect, which is very important in terms of the pathogenesis of many infectious and inflammatory diseases (Riesbeck, 2002; Werber et al., 2005; Potjo et al., 2010; Chen & Chang, 2017). Thus, these antibiotics significantly affect the course of the infectious process. Fluoroquinolones such as ciprofloxacin (second generation), levofloxacin (III generation), moxifloxacin (IV generation) and others are widely used for treatment of patients with severe purulent-inflammatory diseases, in particular those caused by *Staphylococcus aureus* (Kanyuka & Paul, 2011; Dudikova et al., 2015; Paliy et al., 2015). Fluoroquinolones of the second generation are used primarily for the treatment of patients with gram-negative infections caused by *Streptococcus*, mycoplasmas, and *Chlamydia* (Kolios et al., 2006; Majhi et al., 2014; Dudikova et al., 2015; Paliy et al., 2015). Fluoroquinolones of the III and IV generations are highly effective against anaerobes, in particular, moxifloxacin is effective against pneumococci, and prevails over all other fluoroquinolones and antibiotics such as ben-

zylpenicillin, erythromycin, tetracycline, etc. (Werber et al., 2005; Potjo et al., 2010; Chatzika et al., 2014; Majhi et al., 2014; Demir et al., 2018).

Apart from the fact that fluoroquinolones of II–IV generations have a unique mechanism of action, they have a high degree of bactericidal activity, a wide spectrum of antimicrobial action, prolonged half-life, high efficiency in the treatment of infections of any localization, penetrate well into tissues and cells of the body, in particular, they can concentrate in neutrophils and macrophages, with good tolerability and a small percentage of side effects (Ambulkar et al., 2009; Chatzika et al., 2014; Almakaeva et al., 2015; Paliy et al., 2015). The general structural feature of all fluoroquinolones is an oxygen group in position 4, an acidic functional group in position 3, an atom of fluorine in position 6, as well as one substitute in positions 1 and 7. Some fluoroquinolones also have substitutes in the 5 and 8 positions of the basic structure of quinolone (Fig. 1).

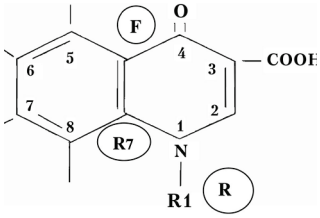

Fig. 1. Chemical structure of fluoroquinolones

At the same time, the substitution in N–1 is obligatory for the antibacterial activity of the molecule. The irreplaceable structures for penetration into the bacterial cell and binding of fluoroquinolones with the enzyme of DNA gyrase are the carboxyl group in position 3 and the keto group in position 4. The fluorine atom in position 6 increases the activity of fluoroquinolone, that is, intensifies the inhibition of DNA gyrase of microorganisms and increases the possibility of antibiotic penetration into the cell.

Since fluoroquinolones have hydrophilic and lipophilic properties, they are often used to treat purulent wound surfaces. They can penetrate into the blood cells, through the wound surface, the mucous membranes, the skin, etc., be carried by blood to various organs and tissues, and thus, as with intravenous infusions, cause a variety of biochemical effects. In this regard, the biological effect of fluoroquinolones is very little studied.

We assume that peripheral blood lymphocytes can also be a convenient and adequate model for studying the mechanism of action of fluoroquinolones, in particular their effect on the arginase-NO-synthase system. This is due to the fact that the intracellular metabolism of the lymphocytes is based on the physiologically and biochemically fixed ability of these cells to respond quickly to any changes of homeostasis in the organism, and modulation of the activity of enzymes in lymphocytes occurs much earlier than their morphological and quantitative parameters (Komisarenko, 2002; Fafula et al., 2012). A number of researchers also believe that peripheral blood lymphocytes may be a convenient, adequate and relevant model for studying physiological and biochemical changes that occur in a number of pathologies (Riesbeck, 2002; Fafula et al., 2012).

To identify pathological processes, physiological and biochemical changes in the organism, in particular those caused by the action of pharmaceuticals, scientists are searching for new biochemical and other markers, trying biochemical and other research methods. In this respect, the role of nitrogen (II) oxide (NO, nitric oxide) as a universal cell and tissue metabolite in the regulation of cellular functions and the paracrine regulator of intercellular and inter-systemic interactions is beyond doubt (Combet et al., 2000; Bryan et al., 2009; Bogdan, 2011; Forstermann & Sessa, 2012).

NO synthesis is carried out from L-arginine,with the participation of NO-synthase (EC 1.14.13.39) by oxidative transformation (Combet et al., 2000; Bryan et al., 2009; Bogdan, 2011; Forstermann & Sessa, 2012). L-arginine is also metabolised with the involvement of arginase. The balance between regulatory physiological and cytotoxic properties is largely due to the local concentration of NO, and the metabolic status of the tissues,where NO synthesizes and realizes its effect (Mielczarek-Puta et al., 2008; Forstermann & Sessa, 2012). At the same time, it is known that, depending on the concentration, the metabolites of the NO cycle can show both pro- and antiproliferative effects (Forstermann & Sessa, 2012). The objective of the present work was studying the effects of fluoroquinolones of the II and IV generations on the activity of the arginase-NO-synthase system of blood lymphocytes.

Materials and methods

The object of research was the blood of practically healthy women. The total number of practically (clinically) healthy women, representative of the age (mean age 53.8 years) was 44. This group was formed from volunteers among the employees of the Danylo Halytsky Lviv National Medical University. The research was approved by the Commission on Bioethics of the Danylo Halytsky Lviv National Medical University (protocol No. 8 of October 22, 2018) and violations of moral and ethical norms during the performance of this work were not revealed. Before turning to study, all patients and healthy donors were made aware of patient information leaflets and gave informed consent to participate in the research. Terms of sample selection meet the requirements of the principles of the Helsinki Declaration on Protection of Human Rights, Convention of Europe Council on Human Rights and Biomedicine and the provisions of laws of Ukraine.

Blood collection by venipuncture was carried out from the median cubital vein in the morning hours under conditions of physiological rest, on an empty stomach, in the amount of 20 mL in test tubes stabilized with heparin (final dilution 1:100). Blood dissolved in a 1:1 with physiological solution, washed in a gradient of density of the Fikoll triumbrast (λ = 1.08 g/cm^3) and centrifuged for 20 minutes at 500 g. The removed interphase rings of mononuclear cells were washed twice within 10 minutes with physiological solution (Boyum, 1968).

After the last centrifugation, a small amount of physiological solution was added to the precipitate, resuspended and using a trypan blue, the count of the number of live and dead cells in the Goryaev camera was performed. The integrity and viability of blood lymphocytes in all experiments was not less than 95%.

For permeabilization of blood lymphocyte membranes and disclosure of latent enzymatic activity, saponin was added to the suspension of blood lymphocytes. This technique is based on previous works on lymphocytes. Blood lymphocytes were incubated for 10 minutes at moderate shaking in a solution containing saponin at a concentration of 0.2% (optimal concentration) (Fafula et al., 2012).

The arginase activity of peripheral blood lymphocytes was determined by the formation of urea, the content of which was measured using a diagnostic kit in accordance with the instructions of the manufacturer (Simko, Ukraine). An enzymatic reaction was initiated by the addition of aliquots (150 μL) of permeabilized saponin lymphocytes to the incubation medium of the following composition (mM): 20 Tris HCl, 100 L-arginine, 2 $MnCl_2$ (pH = 9.5), 300 μl; the amount of protein in the sample was 50–100 μg/mL. The incubation was carried out for 30 minutes at 37 ºC on a shaker. The reaction was stopped by adding 40 μL of 50% trichloroacetic acid to the incubation medium. In control samples, instead of a lymphocyte mixture, an appropriate aliquot of physiological solution was introduced. In addition to experimental and control samples, a sample containing a standard urea solution (16.65 mm) was also prepared. All the specimens were monitored spectrophotometrically at 520 nm. The arginase activity was calculated and expressed as nmol of urea per min per mg of total protein in samples (Shugaley & Kozina, 1977).

The determination of the NO-synthase enzymatic activity of saponin-perforated lymphocytes was carried out at 37 ºC in a medium of incubation in a volume of 1.5 mL of the following composition: tris-HCl – 0.08 M (pH 7.4), $CaCl_2$ – 10 mM, L-arginine – 0.15 mM, NADPH(H^+) – 0.12 mM. Control and non-substratum samples (to which the substrate was not applied) were prepared similarly to the experimental ones, but instead of NADPH(H^+) and L-arginine they contained bi-distilled water. The NO-synthase reaction was initiated by introducing an aliquot of the lymphocyte mixture into the incubation medium (70 μL); the amount of lymphocyte protein in the sample did not exceed 50–70 μg/mL. The ex-

perimental samples were checked spectrophotometrically compared with the control and non-substrate specimens at 340 nm, after which they were incubated for 20 min at 37 °C. The reaction was stopped by adding $HClO_4$ (1.5 M) to the reaction medium (Combet et al., 2000). The NO-synthase activity was expressed in nano-molecules of oxidized NADPH(H^+)/min per 1 mg of total protein in the sample.

The activity of Ca^{2+}-dependent iNOS was determined similarly by adding a selective inhibitor of the inducible isoform aminoguanidine instead of $CaCl_2$ in the incubation medium. The expression of iNOS was induced by adding 0.2 mM H_2O_2 to a suspension of lymphocytes and incubated at 37 ºC for 30 min (Thomas et al., 2007).

Study of kinetic properties of arginase and NO-synthase was performed in a standard incubation medium, which was modified according to the composition of certain components L-arginine (substrate) concentration, incubation time, protein content in the lymphocyte mixture).

The imaginary kinetic parameters characterizing the arginase and the NO-synthase reaction, the initial (instantaneous) reaction rate V_0, maximum amount of the reaction product P_{max}, and the characteristic reaction time were determined as described in the paper (Werber et al., 2005). The imaginary kinetic parameters characterizing the NO-synthase reaction, the apparent contiguity constant to L-arginine KL-Arg, and the maximum reaction rate V_{max} determined by L-arginine, were calculated in the Lineaver-Burke plot.

The results are presented as mean ± standard deviation (x ± SD). Analysis of variance (ANOVA) was used to compare the difference in the means between studied groups. Differences were considered statistically significant at $P < 0.05$ for all analyses.

Results

Arginase is a metalloenzyme that catalyzes the hydrolysis of L-arginine to L-ornithine and urea. Investigation of arginase activity at various pathological conditions of the organism in cells, in particular peripheral blood lymphocytes, is important in terms of understanding the pathogenesis of the disease. There are almost no data on changes in arginase activity with the action of antibiotics of the fluoroquinolone series, which are used for the treatment of a number of bacterial diseases.

When determining the optimal concentrations of substrates for the operation of arginase in peripheral blood lymphocytes and clarifying a number of kinetic parameters for the enzymatic reaction of arginase, L-arginine was introduced into a medium of incubation in the concentration range from 1 to 200 mM (at a constant optimal concentration of Mn^{2+} – 2 mM). At the same time, concentration-dependent growth of the enzymatic activity of arginase was observed, with the output to the plateau at 150 mM concentration of L-arginine (Fig. 2). Specificaly, arginase activity was 1.44 times ($P < 0.05$) higher under the effect of ciprofloxacin, 1.52 times ($P < 0.05$) higher under effect of levofloxacin and 1.65 ($P < 0.001$) times higher under the effect of moxifloxacin compared to arginase activity in untreated cells.

Based on linearization of the data in the Lineweaver-Burke plot (Fig. 3), a mixed type of inhibition of the enzyme activity is shown and the main kinetic parameters of L-arginine metabolism by saponin-permeabilized blood lymphocytes of the control group and under the influence of fluoroquinolones are determined (Table 1).

Table 1
Kinetic parameters characterizing hydrolysis of L-arginine by saponin-permeabilized blood lymphocytes under effect of fluoroquinolones (x ± SD, n = 6)

Kinetic parameters	Control	Antibiotics		
		ciprofloxacin, 10^{-5}M	levofloxacin, 10^{-5}M	moxifloxacin, 10^{-5}M
V_{max}, nmol urea/min·mg protein	147.1 ± 12.2	212.8 ± 19.3*	238.1 ± 20.4*	285.2 ± 23.2**
$K_{L\text{-}arg}$, mM	27.3 ± 2.1	40.2 ± 3.7*	43.9 ± 3.8*	57.5 ± 4.2**

Note: V_{max} – maximum reaction rate of arginase, $K_{L\text{-}Arg}$ – Michaelis constant (affinity) to L-arginine, changes are significant compared to the values of the control group; * – $P < 0.05$, ** – $P < 0.001$.

The calculation of the kinetic parameters of arginase activity indicates that the maximum rate (V_{max}) of the metabolism of L-arginine by saponin-permeabilized blood lymphocytes under the action of ciprofloxacin was 1.40 ($P < 0.05$), levofloxacin was 1.60 ($P < 0.05$) and moxifloxacin was 1.94 ($P < 0.001$) times higher than control values. The apparent affinity constant of arginase to L-arginine (KL-arg) in lymphocytes in the blood increased with ciprofloxacin by 1.52 ($P < 0.05$), levofloxacin by 1.60 ($P < 0.05$), and moxifloxacin by 2.14 ($P < 0.001$) times compared to this value in lymphocytes of control group. It was shown that arginase activity of blood lymphocytes of practically healthy persons (physiological norm) with optimal concentrations of arginine (150 mM) and in the absence of fluoroquinolones was 124.2 ± 8.8 nmol of urea/min per 1 mg of protein (Fig. 4). Under the effects of antibiotics of the fluoroquinolone series on blood lymphocytes at concentrations from 10^{-5} to 10^{-2} M, arginase activity increased in dose-dependent manner. At concentrations of the investigated fluoroquinolones 10^{-5} M this increase for ciprofloxacin was 1.92 times ($P < 0.001$), for levofloxacin 2.16 times ($P < 0.001$), and for moxifloxacin 2.41 times ($P < 0.001$) compared to untreated cells.

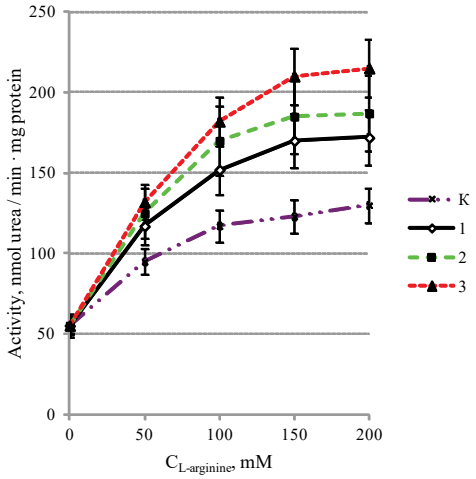

Fig. 2. The dependence of arginase activity of blood lymphocytes on the concentration of the substrate (L-arginine) in the incubation medium and the influence of fluoroquinolones (10^{-5} M): *K* – control, *1* – ciprofloxacin, *2* – levofloxacin, *3* – moxifloxacin; x ± SD, n = 6

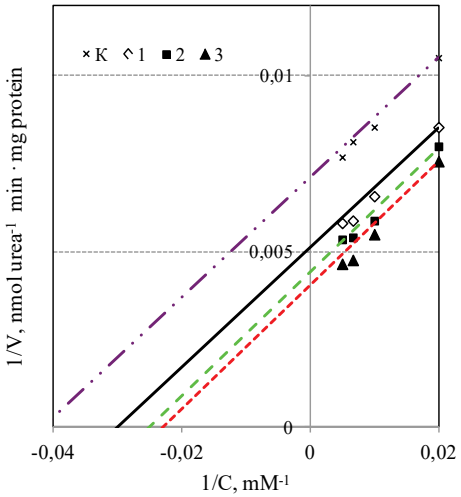

Fig. 3. Linearization of concentration-dependent curves of arginase activity in blood lymphocyte on the substrate concentration in the incubation medium and on the effect of fluoroquinolones (10^{-4} M) in the Lineweaver-Burke plot: *1* – ciprofloxacin, *2* – levofloxacin, *3* – moxifloxacin; x ± SD, n = 6

Since L-arginine is a substrate not only for arginase, but for all isoforms of NO-synthase, the next step was to study the activity of certain isoforms of NO-synthase and their kinetic parameters under the influence of fluoroquinolones.

As it is known, all isoforms of NOS have been identified in blood lymphocytes. As a result of the conducted research, it was found that cNOS activity of blood lymphocytes of practically healthy women is 71.4 ± 6.9 nmol of NADPH(H^+)/min per 1 mg of protein (Fig. 5). Analysis of published data shows the significant variability of absolute values of enzyme activity of NOS blood lymphocytes, which is probably due to the variety of methodological approaches to the study of enzyme activity.

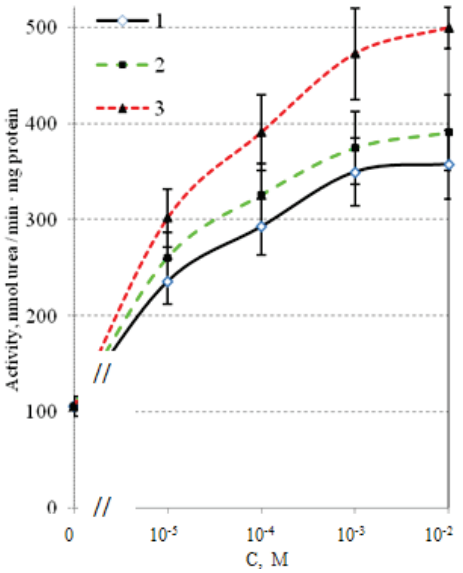

Fig. 4. Influence of the concentration of antibiotics of different generations: ciprofloxacin (*1*), levofloxacin (*2*) and moxifloxacin (*3*) on arginase activity of peripheral blood lymphocytes: changes are significant at the concentration of antibiotics 10^{-5}–10^{-2} M compared to the values of the control group; P < 0.001; x ± SD, n = 6

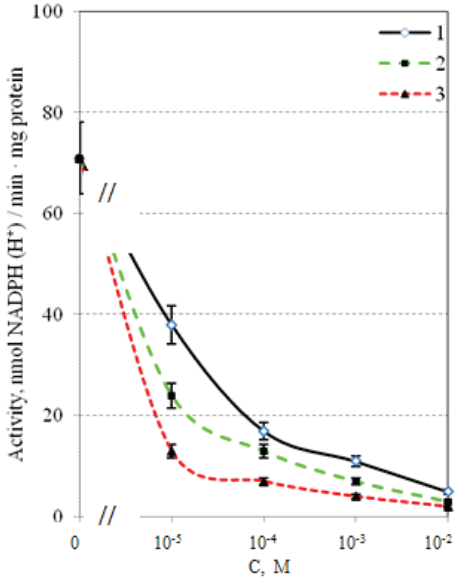

Fig. 5. Influence of concentration of antibiotics of different generations: ciprofloxacin (*1*), levofloxacin (*2*) and moxifloxacin (*3*) on cNOS activity of peripheral blood lymphocytes: changes are significant at the concentration of antibiotics 10^{-5}–10^{-2} M compared to the values of the control group, P < 0.001; x ± SD, n = 6

In blood lymphocytes under the influence of the studied fluoroquinolones in the concentration of 10^{-5} M, ciprofloxacin caused a decrease in the activity of cNOS by 1.95 times (P < 0.001), levofloxacin – by 3.04 times (P < 0.001) and moxifloxacin – by 5.46 times (P < 0.001) compared to cNOS activity in blood lymphocytes of the control group (P < 0.001).

It has been established that the iNOS activity of blood lymphocytes in practically healthy women was identified to a small extent, practically at the margin of error, and was 1.58 ± 0.18 nmol of NADPH(H^+)/min per 1 mg of protein (Fig. 6).

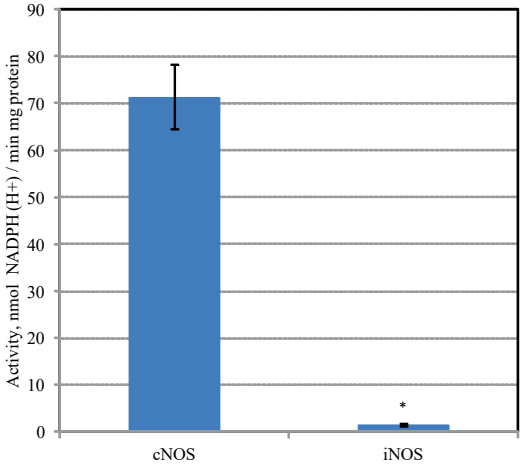

Fig. 6. Comparative values of the constitutive and inducible isoform NO-synthase activity in blood lymphocytes at physiological norm: * – P < 0.001 compared to cNOS activity; x ± SD, n = 44

When studying the influence of fluoroquinolones on the activation of iNOS in lymphocytes isolated from the blood of practically healthy women, we did not observe its activation, and the inhibitory effect could not be determined due to its low enzyme activity. Oxidative stress was used to induce iNOS activity in blood lymphocytes, preincubating lymphocytes with H_2O_2. Preincubation of lymphocytes with 0.2 mM H_2O_2 led to an increase in iNOS activity by 31.30 times, from 1.58 to 34.3 nmol NADPH (H^+)/min per 1 mg of protein (Fig. 7). By activation of iNOS with hydrogen peroxide, cyprofloxin led to inhibition of enzyme activity by 1.22 times (P < 0.05), levofloxacin by 1.45 times (P < 0.05), and moxifloxacin by 2.34 times (P < 0.001).

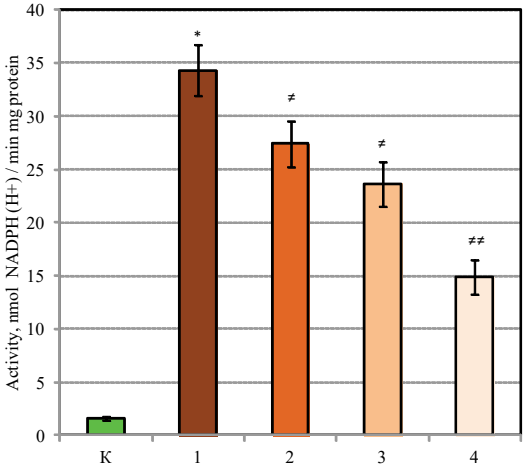

Fig. 7. Influence of fluoroquinolones (10^{-5} M) on the activity of inducible isoform NO-synthase in blood lymphocytes under conditions of H_2O_2-induced stress: *K* – control; *1* – at the presence of H_2O_2 in an incubation medium; *2* – H_2O_2 + ciprofloxacin; *3* – H_2O_2 + levofloxacin; *4* – H_2O_2 + moxifloxacin, * – P < 0.001 compared to iNOS activity at control, $^{\neq}$ – P < 0.05, $^{\neq\neq}$ – P < 0.001 compared to iNOS activity without fluoroquinolones in an incubation medium; x ± SD, n = 6–8

Thus, the increase in the activity of arginase in blood lymphocytes under the action of fluoroquinolones indicates the general need for L-arginine by cells. Changes in the activity of arginase and NOS enzymatic systems only indicate the direction of dysmetabolic disturbances in the system of NO-homeostasis. However, the biochemical mechanisms leading to changes in the functional activity of the investigated enzymatic systems under the influence of fluoroquinolones remain unclear. There-

fore, the next stage of our research was to study of kinetic properties of cNOS and iNOS isoforms of blood lymphocytes.

In order to study the mechanisms of functioning of NO-synthase, the initial (instantaneous) reaction rate (V_0), the maximum formation of the reaction product (P_{max}) and the characteristic reaction time were determined. To determine these kinetic parameters of NOS, the dynamics of reduction of NADPH(H^+), which indicates the synthesis of NO, were studied. For this, the suspension of lymphocytes was incubated in a standard incubation medium for various time intervals from 0–30 min. The results of studies show that the kinetic curves of NO formation in the process of NO-synthase reaction of blood lymphocytes tend to saturate (Fig. 8). From this figure it can be seen that the kinetics of the formation of NO, with the participation of cNOS, is consistent with the laws of the reaction of zero order in the range 0–20 min: in this interval of time the dependence of the formation of NO on the incubation period is practically linear. Therefore, in subsequent experiments, the duration of incubation of lymphocytes and, accordingly, NO-synthase reaction was 20 minutes.

The dynamics and amount of NO formation with the participation of cNOS of blood lymphocytes under the influence of fluoroquinolones are significantly lower compared to values in untreated cells (Fig. 8).

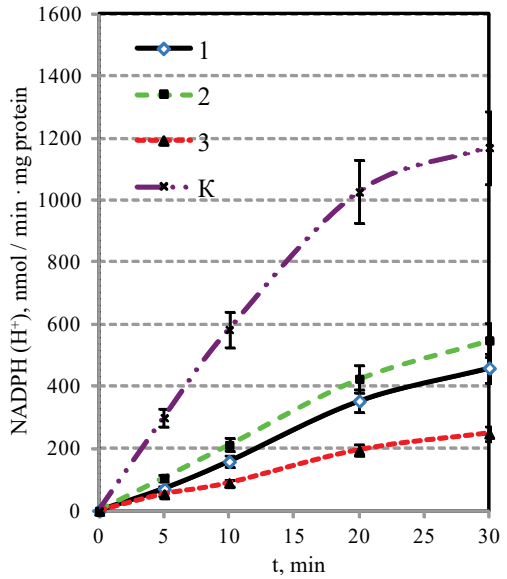

Fig. 8. Dynamics of NADPH(H^+) consumption in the process of cNO-synthase reaction of blood lymphocytes under the influence of fluoroquinolones: x ± SD, n = 6

By linearizing the obtained data in coordinates {P/t on P}, the basic kinetic characteristics of NOS of blood lymphocyte reaction are calculated (Table 2). The values of the kinetic parameters for cNOS of lymphocytes in the control group and under the influence of fluoroquinolones differ significantly. Thus, initial (instantaneous) reaction rate in the control group was 92.2 ± 7.1 nmol NADPH(H^+)/min per 1 mg of protein. Under the effect of ciprofloxacin, initial (instantaneous) reaction rate decreased significantly by 3.25 times ($P < 0.001$), by 3.42 times ($P < 0.001$) with levofloxacin, and by 4.95 times ($P < 0.001$) with moxifloxacin.

Table 2
Kinetic parameters of constitutive NO-synthase reaction of blood lymphocytes under the influence of fluoroquinolones (x ± SD, n = 6)

Parameters	Control	Ciprofloxacin, 10^{-5} M	Levofloxacin, 10^{-5} M	Moxifloxacin, 10^{-5} M
V_0, nmol NADPH(H^+)/ min·mg protein	92.2 ± 7.1	28.8 ± 2.9**	27.4 ± 3.1**	18.8 ± 1.3**
P_{max}, nmol NADPH(H^+)/ mg protein	3408 ± 285	961.2 ± 85.3**	944.3 ± 87.4**	883.5 ± 77.4**
τ, min	36.1 ± 4.0	41.6 ± 4.2	43.7 ± 4.1	50.2 ± 4.3*

Note: V_0 – the initial (instantaneous) reaction rate, P_{max} – the maximum formation of the reaction product, τ – the characteristic reaction time, changes are significant compared to the values of the control group; * – $P < 0.05$, ** – $P < 0.001$.

Regarding the kinetic parameters of the inducible NO-synthase reaction, due to its low activity in the control, it was not possible to calculate them correctly. The NO production with the participation of cNOS under the influence of fluoroquinolones is slower and less active than in their absence. Regarding the maximum amount of the reaction product produced in the cNO-synthase reaction, under the effect of ciprofloxacin, it was formed 3.58 times less than in the control group ($P < 0.001$). Under the effect of levofloxacin it was 3.60 times less, and under action of moxifloxacin 3.84 times less than in the control group. Analysis of the characteristic reaction time showed that it increased from 36.1 min (control) to 50.2 min ($P < 0.05$).

Discussion

It was found that throughout the range of the studied concentrations of L-arginine, the arginase activity with fluoroquinolones increased compared to that in the control group. Enzyme activity increased in the following sequence: control → ciprofloxacin → levofloxacin → moxifloxacin. When studying the influence of fluoroquinolones on the arginase activity it is shown that these data correlate directly with generations of fluoroquinolones. Ciprofloxacin belongs to the second generation, levofloxacin – to the third generation. The highest enzyme activity was observed under the action of moxifloxacin, which belongs to the IV generation (Dudikova et al., 2015).

With the interpretation of the data obtained, determined by L-arginine, it is possible to conclude that increase of arginase activity in lymphocytes with the influence of fluoroquinolones occurs due to the growth of the turnover number of enzymes (V_{max} increases), although the affinity of enzyme to the substrate decreases (KL-argincreases). Thus, the obtained results prove that with the influence of fluoroquinolones the arginase activity of peripheral blood lymphocytes increases significantly, which is primarily due to the growth of the maximum reaction rate.

On the basis of the study of the activity of NO-synthases, it can be assumed that such a significant decrease in the cNOS activity in blood lymphocytes may be a marker for the effects of fluoroquinolones. It is known that cNOS produces low NO concentrations, while iNOS synthesizes high concentrations of NO (> 300 nM) (Bryan et al., 2009; Forstermann & Sessa, 2012). iNOS is a calcium-independent isoform of NOS and, unlike cNOS, is not expressed continuously (constitutively).

It should be noted that similar results concerning the inhibition of iNOS by fluoroquinolones have been obtained on other objects. Thus, it has been shown that moxifloxacin inhibits iNOS in epithelial cells of the human respiratory tract (Majhi et al., 2014). Stimulation of cells by cytokines leads to an increase of the level of NO by 3.30 times and moxifloxacin inhibits this increase by 68%. Also, the intensification of iNOS activity was inhibited by 62% with the addition of moxifloxacin (Majhi et al., 2014). Similar results regarding inhibition of iNOS by ciprofloxacin have been obtained *in vivo* due to the treatment of multidrug-resistant *Streptococcus* pneumonia also (Werber et al., 2005). Significant decrease of NO 8 was observed after 8 hours and lasted up to 24 hours after antibacterial therapy.

As far as microbiological characteristics are concerned, ciprofloxacin (II generation) is a "golden standard" among fluoroquinolones. Its bioavailability is 80%. It works mainly on gram-negative flora and on some gram-positive microorganisms. Its action on *E. coli*, *Salmonella*, *Shigella* is significant. Among gram-positive microorganisms, the most sensitive are staphylococci and mycobacteria tuberculosis. Moderate activity is shown in relation to pneumococci, enterococci, chlamydiae.

Levofloxacin (III generation) has advantages over other fluoroquinolones in its activity against pneumococci, *Mycoplasma*, chlamydiae. Bioavailability for peroral reception is 100%. Besides respiratory infections, it is used to treat infections of the genitourinary system, skin, soft tissues. Moxifloxacin (IV generation) exhibits significantly higher activity in relation to gram-positive microorganisms than the above mentioned fluoroquinolones. It is highly active against streptococci, staphylococci and, to a lesser extent, enterococci. The activity of moxifloxacin in

relation to pneumococci is 4–16 times higher than that of cyprofloxacin. The drug is effective against gram-positive bacteria resistant to many other antibiotics. It also has high activity in relation to gram-negative bacteria, in particular enterobacteria, nitrobacteria, gonococci. Moxifloxacin also acts on *Chlamydia*, *Mycoplasma*, *Ureaplasma*, *Mycobacterium tuberculosis*. However, it is inferior to levofloxacin in action on the blue pus bacillus. The bioavailability of this drug when taken orally is 90%. In general, the literature sources suggest that moxifloxacin is the most effective and safe antibacterial drug among other fluoroquinolones, in particular for the treatment of respiratory infections. It has a wide range of antimicrobial activity, very positive pharmacokinetic properties, high clinical efficiency at upper and lower respiratory tract infections, and good tolerability.

Thus, while the antimicrobial properties of fluoroquinolones have been studied quite well, their parallel action on the biochemical processes and regulatory mechanisms of the cells of the body is almost uninvestigated. The results we obtained concerning the action of fluoroquinolones indicate a disorder of the arginase-NO-synthase system of blood lymphocytes, which leads to disbalance of lymphocytes regulatory systems, in particular the regulatory function of NO. Reduction of iNOS activity with the action of fluoroquinolones by the induced H_2O_2, suggests that these antibiotics can prevent hyperproduction of NO in blood lymphocytes. It is known that NO, produced in excessive amounts in pathological conditions of the body, has a significant cytotoxic effect due to the formation of peroxynitrite (the product of the interaction between NO and superoxide anion radical), which can destroy almost all of the cell components (Thomas et al., 2007; Forstermann & Sessa, 2012). It is likely that one of the mechanisms of action of fluoroquinolones is the reduction of hyperproduction of NO. Under physiological conditions, arginase regulates the concentration of L-arginine in a cell and inhibits the activity of NOS by competing for a common substrate, and thus directly regulates the synthesis of NO (Mielczarek-Puta et al., 2008; Forstermann & Sessa, 2012).

L-arginine is the only substrate for the synthesis of NO by all the forms of NOS. The availability of intracellular L-arginine is a limiting factor for NO synthesis and a potential mechanism for controlling the regulatory function of NO, since most types of cells cannot synthesize L-arginine and require its exogenous intake. L-arginine acts as a key molecule in a number of other metabolic, as well as regulatory and signaling, pathways, undergoing serious changes in various pathological conditions (Mielczarek-Puta et al., 2008).

Conclusion

When interpreting the obtained kinetic parameters determined by L-arginine, it has been shown that fluoroquinolones increase arginase activity in lymphocytes by increasing the turnover number of enzymes (V_{max} is increasing) at decreasing the enzyme affinity to the substrate (KL-arg increases). The obtained kinetic parameters indicate that in the blood lymphocytes under the influence of fluoroquinolones the synthesis of NO with the participation of cNOS is inhibited and NO hyperinduction is inhibited due to activation of iNOS, which is characteristic for pathological conditions. Consequently, under the influence of fluoroquinolones on blood lymphocytes, the ratio of NO-synthase and arginase metabolism of L-arginine is disturbed, indicating distributional changes in the synthesis of NO.

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