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QUERCETIN AND HISTAMINE EFFECTS ON THE CONTENT OF SUPEROXIDE ANION AND ATP IN THE BLOOD PLASMA OF RATS

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Background. Histamine is one of the versatile biogenic amines with multiple roles in the immune response and allergic disorders. Histamine and ATP can act as ligands in the body. In plasma, ATP is a potent vasodilator that stimulates the formation of NO and prostaglandins and, very importantly, can offset local sympathetic vasoconstriction. Adenosine triphosphate is released into plasma from erythrocytes and endothelial cells, and the plasma concentration increases in both the feed artery and the vein draining the contracting skeletal muscle. Taking this into account, it is important to study the effect of histamine in combination with quercetin, which inhibits the release of histamine from cellular depots, on the content of the superoxide anion and ATP in the blood plasma.

Materials and Methods. Nonlinear white male rats were used for the experimental studies. Quercetin solutions were added to whole blood to a final concentration of 0.1; 0.3; 0.5; 1; 3; 5 mM. In other experiments, histamine solution was added to the blood to final concentration of 0.01; 0.1; 1; 10 μM. In a series of experiments, histamine (0.01 and 10 μM) and quercetin (0.1; 0.5; 3; 5 mM) were added to the blood in various possible combinations. Blood plasma was used in each experimental group. The blood to which saline was added was used as control. The content of superoxide anion and adenosine triphosphate was measured in the selected samples.

Results and Discussion. Histamine, quercetin, as well as their combined action lead to the intensification of superoxide anion generation in the blood plasma of rats.

Quercetin is known to be an antioxidant, but some of our studies have shown the opposite effect. Quercetin causes an increase in the ATP content in the blood plasma of rats. Histamine causes the same effect only at concentrations of 0.1 and 10 μM. Likewise, the combined administration of histamine and quercetin into the blood increased the ATP content. The results of ANOVA test prove that both histamine and quercetin have the same effect on the release of superoxide anion and ATP from blood cells into plasma. Their combined action only strengthens the effect of releasing the studied products.

Conclusion. Quercetin, histamine and their combined action cause an increase in the generation of superoxide anion and ATP in the blood plasma of rats.

Keywords: quercetin, histamine, superoxide anion, ATP

INTRODUCTION

Histidine can be decarboxylated to histamine by histidine decarboxylase (Brosnan *et al.,* 2020; Zhao *et al.,* 2022). Histamine is one of the most versatile biogenic amines with multiple roles during the immune response and in allergic disorders. With four distinct GPCRs (histamine H1–H4 receptors), intracellular binding sites (most likely members of the cytochrome P450 family) as well as a membrane transporter (organic cation transporter) expressed in various immunocompetent cells, histamine can induce a complex network of interactions. These signaling pathways are expressed differently depending on the stage of differentiation or activation of target cells, thus adding a further degree of complexity to the system (Číž & Lojek, 2013; Nazar *et al.,* 2021). For example, TRPC6 (transient potential-controlled receptor-6) is a cation-selective, diacylglycerol-regulated, Ca^{2+}-permeable channel that is activated by agonists of Gq proteins. TRPC6 dysfunction is associated with the pathogenesis of various cardiovascular and renal diseases, such as vasospasm and glomerulosclerosis. When stimulated by agonists of the H1 receptor, the activity of TRPC6 decreases over time to the initial level, despite the constant presence of the agonist. Protein kinase-dependent phosphorylation of the H1 receptor leads to a decrease in the formation of intracellular diacylglycerol, which contributes to the regulation of TRPC6 activity (Chen *et al.,* 2014). Histamine is generated by several cells during the immune response not only through the release of intracellular stores in mast cells or basophils in response to IgE-dependent or – independent stimulus, but also through de novo synthesis, catalyzed by histidine decarboxylase, in a number of haemapoietic cells that secrete the amine immediately without prior storage. These features enable histamine to tune the fine balance between immunity and tolerance by affecting the polarization and cytokine production of dendritic cells, immunoregulatory cells, natural killer cells, epithelial cells, B-lymphocytes and T-lymphocytes, thus providing new pharmacological strategies to control immune reactivity during immune disorders, such as autoimmunity. The discovery, at the turn of the millennium, that the histamine H4 receptor is largely expressed in haematopoietic cells as well as its chemotactic properties suggest that it has a regulatory role in the immune system. Histamine H4 receptors modulate eosinophil migration and selective recruitment of mast cells, leading to an amplification of histamine-mediated immune responses and eventually to chronic inflammation. The involvement of histamine H4 receptors in dendritic cell activation and T cell differentiation demonstrate that it has an immunomodulatory function (Číž & Lojek, 2013; Nazar *et al.,* 2021). It is believed that the cells of the body express all four types of histamine receptors, but their number in different cells is not the same

(Radchenko, 2017). The ratio of receptor types in immunocompetent and hematopoietic cells is still unknown.

The *in vitro* effects of histamine on the chemiluminescence response of bovine neutrophils were determined by T. R. Phillips *et al.* (1987); the addition of histamine was found to significantly suppress the chemiluminescence response of these neutrophils. This suppression was dependent on the continuous presence of histamine in the culture media. Hydrogen peroxide-generated chemiluminescence was also suppressed by high concentrations of histamine. The results of this study suggest that histamine has a pharmacological or regulatory role in the control of the oxidative burst reaction of bovine neutrophils. Researchers concluded that high concentrations of histamine (from 10^{-5} to $5{\cdot}10^{-3}$ M) stimulated the neutrophils to produce the active forms of oxygen via histamine H1 receptors and the NADPH oxidase pathway (Číž & Lojek, 2013; Nazar *et al.,* 2021). It should be noted that determining the content of histamine in blood or tissues has no diagnostic value, since this biogenic amine is quickly released during inflammation or an allergic reaction, stimulates histamine receptors and triggers a cascade of corresponding reactions, while the excess of histamine begins to be neutralized by histamine and histamine-*N*-methyltransferase. The elimination of histamine takes about 15 minutes (Radchenko, 2017).

Histamine and ATP can act as ligands in the body. For example, in the adult human chondrocyte (and at least one cell line, OUMS-27, a model for chondrocyte cell physiology), a significant regulatory paradigm for intracellular Ca^{2+} homeostasis and definition of cell phenotype can be described as a feedback loop. Ligand (ATP or histamine) triggered release of Ca^{2+} from one or more intracellular stores (Suzuki *et al.,* 2020). In plasma, ATP is a potent vasodilator that stimulates the formation of NO and prostaglandins and, very importantly, can offset local sympathetic vasoconstriction. Adenosine triphosphate is released into plasma from erythrocytes and endothelial cells, and the plasma concentration increases in both the feed artery and the vein that drains the contracting skeletal muscle (Mortensen & Saltin, 2014). Femoral arterial and venous [ATP] values were 109 ± 34 and 147 ± 45 nmol L^{-1} at rest and increased to 363 ± 83 and 560 ± 111 nmol L^{-1}, respectively, during exercise. Hypoxia increased venous plasma [ATP] at rest compared to normoxia, but not during exercise. Arterial ATP infusion (≤1.8 μmol min^{-1}) increased arterial plasma [ATP] from 74 ± 17 to 486 ± 82 nmol L^{-1} (Mortensen *et al.,* 2011). Purinergic signaling involves the activation of cell surface P1 and P2 receptors by extracellular nucleosides and nucleotides such as adenosine and adenosine triphosphate (ATP), respectively. P2 receptors comprise P2X (in particular P2X1 and P2X7) and P2Y (in particular P2Y1 and P2Y12, P2Y13) receptors, and have well-established roles in leukocyte and platelet biology. Emerging evidence indicates important roles for these receptors in red blood cells. P2 receptor activation stimulates a number of signaling pathways in progenitor red blood cells resulting in microparticle release, reactive oxygen species formation, and apoptosis. Likewise, activation of P2 receptors in mature red blood cells stimulates signaling pathways mediating volume regulation, eicosanoid release, phosphatidylserine exposure, hemolysis, impaired ATP release, and susceptibility or resistance to infection (Sluyter, 2015). P2Y-purinoreceptors and M3-cholinergic receptors are connected to their effector enzymes through the same type of G-proteins (Melenevska *et al.,* 2007).

Quercetin (3,31,41,5,7-pentahydroxyflavone), a naturally occurring polyphenol flavonoid, found in some fruits and vegetables, including onions, capers, apples, berries,

tea, tomatoes, grapes, Brassica vegetables, and shallots, as well as many nuts, seeds, barks, flowers, and leaves (Di Petrillo *et al.,* 2022). Quercetin is a flavonoid which is rich in antioxidants. Quercetin belongs to the fourth class of bioavailability (Kovalevska, 2014). It has anti-allergic functions that are known for inhibiting histamine production and pro-inflammatory mediators (Mlcek *et al.,* 2016). Quercetin can regulate the Th1/Th2 stability, and decrease the antigen-specific IgE antibody releasing by B cells. Quercetin plays a major role in antiinflammatory and immunomodulatory function which makes it proper for the management of different diseases. The plasma level of quercetin is normally in low ranges, but after consuming foods that are highly rich in it, the plasma's level of it increases to different ranges. Quercetin contains 3 rings and 5-hydroxyl group and is naturally found in plants as a glycone or carbohydrate conjugate. There are several potential benefits of quercetin in terms of total health and disease resistance such as its anti inflammatory and antioxidant effects, as well as the ability to inhibit lipid peroxidation, platelet aggregation and capillary permeability. Quercetin inhibits lipopolysaccharide (LPS)-induced tumor necrosis factor alpha (TNF-α) production in macrophages, LPS-induced interleukin (IL)-8 production in lung A54 cells, LPSinduced mRNA levels of TNF-α and IL-1α in glial cells, production of inflammation-producing enzymes (cyclooxygenase and lipoxygenase), and FcεRI-mediated release of proinflammatory cytokines, tryptase, and histamine from human umbilical cord blood-derived cultured mast cells (Morteza *et al.,* 2020).

Taking this into account, it is important to study the effect of histamine in combination with quercetin, which inhibits the release of histamine from cellular depots (Mlcek *et al.*, 2016; Jafarinia *et al.,* 2020; Di Petrillo *et al.,* 2022), on the content of the superoxide anion and ATP in the blood plasma.

Therefore, the purpose of the study was to analyze the changes in the content of free radicals and ATP in the blood plasma of rats under the action of histamine and quercetin.

MATERIALS AND METHODS

The nonlinear white male rats with body weight of 180–220 g were used for the experimental studies (*Rattus norvegicus* f. *Domesticus*). Chloroform was used for euthanasia. Animals were treated in accordance with the requirements of the European Convention for the Conservation of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg, France 1986) and in accordance with the General Principles of Animal Work approved by the First National Congress on Bioethics (Kyiv, Ukraine, 2001) and also approved by the Ethics Committee of Ivan Franko National University of Lviv, Ukraine (protocol No. 31-02-2023). After decapitation of the animals, blood was collected in a glossy cup with heparin. An adequate amount of quercetin solutions was added to whole blood to a final concentration of 0.1; 0.3; 0.5; 1; 3; 5 mM (0.01 mL of 0.01 M, 0.03 M, 0.05 M, 0.1 M, 0.3 M, 0.5 M quercetin solution was added to 1 mL of blood). Concentrations of 1 and 3 mM are therapeutic and used in pharmacy (single concentration for oral administration). Quercetin (brand PFA) was dissolved in warm saline at 37 °C (oral administration of quercetin dissolved in warm water is used in medicine). In other experiments, a histamine solution (0.01% histamine dihydrochloride solution used as a stock solution; manufacturer – Limited Liability Company "Immunolog", Ukraine, Vinnytsya) was added to the blood to final concentration of 0.01; 0.1; 1; 10 μM (0.01 ml of 10^{-6} M, 10^{-5} M, 10^{-4} M, 10^{-3} M histamine solution was added

to 1 mL of blood). 0.9% NaCl was used to prepare the solutions. In a series of experiments, histamine (minimum and maximum test concentrations: 0.01 and 10 μM) and quercetin (at a concentration of 0.1; 0.5; 3; 5 mM) were added to the blood in various possible combinations. For this Quercetin concentrations were chosen as follows: minimum and maximum concentrations, where 0.5 and 3 mM are those concentrations that did not change the content of lipid hydroperoxides in plasma of blood in our previous *in vitro* experiments (Harasym *et al.,* 2020). Blood plasma of five rats (n = 5) was used in each experimental group. The blood samples to which saline was added were used as control (0.01 mL of physiological solution was added to 1 mL of blood). The blood with physiological solution was incubated for 5 min, then centrifuged at 3000 rpm for 10 min. The experiment was performed at room temperature of 19 °C. Blood plasma, selected for analysis, was laundered. The content of superoxide anion ($O_2^{-\bullet}$; Denisenko & Kostenko, 2002) and adenosine triphosphate (ATP; Ushakova & Dyomshina, 2015) was measured on the spectrophotometer ULAB102UV (China) in the selected samples. Protein content was determined by Lowry assay (Lowry, 1951). Analyses of data were conducted in Excel-10 for Windows (Descriptive statistics, Two-way ANOVA) and SPSS (Shapiro–Wilk test, One-way ANOVA). The values of parameters are given as a mean (M) ± standard error (m) and the degree of probability of difference (p) between indicators, where appropriate. The ANOVA test was performed to determine the statistical significance (Two-way ANOVA with the post-hoc Tukey test). The difference was considered significant at the confidence index $p \geq 0.95$; $p \geq 0.99$; $p \geq 0.999$. We checked the general population of data for normal data distribution (Shapiro–Wilk test) and homogeneity of intragroup variances (Leven's test). The results of the verification allowed us to use parametric methods of statistical analysis to compare experimental groups and conduct Two-way ANOVA.

RESULTS AND DISCUSSION

The effect of histamine and quercetin on the content of superoxide anion in the blood plasma of rats. For *in vitro* experiment, we reached the appropriate concentration of quercetin or histamine by adding them to whole blood. The work solutions were prepared by dissolving the examined substances in isotonic saline to preserve erythrocytes (to prevent hemolysis). This study aimed to estimate quercetin and histamine effects on the superoxide anion content in blood cells, as it is known that these substances have a significant impact on the physicochemical and biochemical parameters of blood.

Superoxide is a primary oxygen radical that is produced when an oxygen molecule receives one electron. Superoxide anion plays a fundamental role in oxidative stress and oxidative damage in biological systems. The enzyme superoxide dismutase rapidly neutralizes the superoxide radical in the body (Shalai, 2020).

We observed that the content of superoxide anion increased directly proportionally from 56 to 372 times with an increase in the concentration of quercetin in the blood plasma of rats (**Fig**. **1**). Thus, in the control samples, the content of superoxide anion was equal to 0.0007 ± 0.00005 nM/second·mg of protein. At a quercetin concentration of 0.1 mM, the content of superoxide anion was 0.039 ± 0.003 nM/second·mg of protein, and at the maximum concentration of quercetin (5 mM), the amount of the studied product was 0.26 ± 0.009 nM/second·mg of protein.

D. Xu *et al.* testify that, when quercetin is applied at high doses, the dynamic balance of GSH (under the action of GSH peroxidase) is affected; H_2O_2 is converted to H_2O and GSH is oxidized to GSSG (oxidized glutathione disulfide). GSH reductase catalyzes the reduction of GSSG in the liver and red blood cells (by providing H) to form GSH. Thus, the dynamic balance of GSH is produced, which may cause the inhibition of GSH levels in low doses (Xu *et al.,* 2019). The decrease in the content of reduced glutathione (established by our previous studies (Harasym *et al.,* 2021) leads to the accumulation of free radicals in the plasma, in particular superoxide anion (in our case).

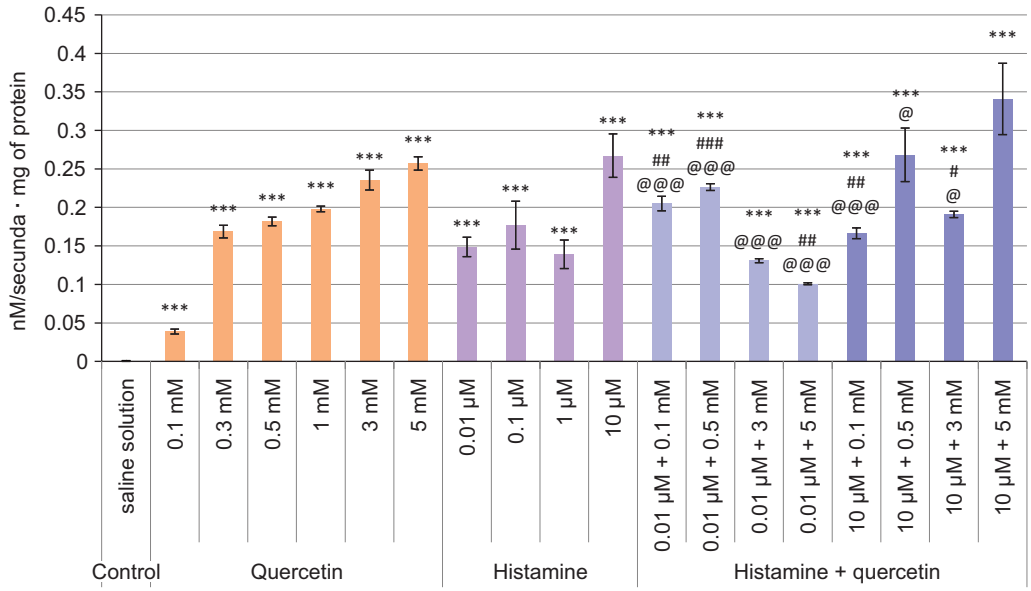

Fig. 1. The content of superoxide anion in blood plasma of rats under the action of quercetin, histamine and the combination of histamine and quercetin (** – p ≥0.99; *** – p ≥0.999; * – compared to control; # – compared to the effect of histamine of the corresponding concentration; @ – compared to the effect of quercetin of the corresponding concentration)

K. Griffiths *et al.* report that flavonoids have a protective effect on the DNA damage caused by hydroxyl radicals, presumably through chelating metal ions, such as copper or iron. The flavonoids, complexed with copper or iron, prevent the generation of reactive oxygen species. However, some flavonoids such as quercetin may have either a protective or damaging effect of reactive oxygen species (due to $HO^{\bullet}$, $O_2^{-\bullet}$, $HOO^{\bullet}$) upon DNA, depending on the concentration of chelating metal ions (Griffiths *et al.,* 2016). A dose-dependent increase in the content of superoxide anion probably occurs due to the high concentration of the quercetin solutions we used. These concentrations are optimal for oral use (*in vivo* conditions). When we inject such concentrations directly into the blood, quercetin shows a harmful effect. Flavonols (to which quercetin belongs) have a gallic structure and therefore can auto-oxidize with the formation of superoxide anion (Hodnick *et al.,* 1986). L. M. Ruiz *et al.* prove that quercetin uncouples oxidation and phosphorylation in mitochondria and increases the formation of superoxide anion (Ruiz *et al.,* 2015).

Histamine also leads to an increase in the superoxide anion content. Histamine, at a concentration of 10 μM, markedly influenced the amount of the studied product in the blood plasma (inreased in 386 times). Histamine with dosages of 0.01 μM, 0.1 μM and 1 μM increased the content of superoxide anion by 215, 256, and 201 times (**Fig. 1**). Noteworthy, histamine with a maximum dosage leads to the most intensive increase in the content of superoxide anion. Combitantion of histamine with dosage of 10 μM and quercetin with dosages of 0.1 and 3 mM reduced the content of superoxide anion compared to histamin alone, but the level of the studied indicator still exceeded the control values (**Fig. 1**). With the combined effect of histamine at a concentration of 10 μM and quercetin at a concentration of 0.5 and 5 mM, there was no decrease in the superoxide anion content compared to plasma samples to which only histamine at a concentration of 10 μM was added. Therefore, quercetin with a minimum dosage (0.1 mM) and therapeutic dosage (3 mM) positively acts on the generation of superoxide anion combined with the influence of histamine with a maximum dosage.

Our results show that quercetin at low concentrations (0.1 and 0.5 mM) combined with the action of histamine at a minimal concentration (0.01 μM) led to an intensification of the accumulation of superoxide anion content in the blood plasma of rats compared to the effect of histamine alone at an appropriate concentration, which was a negative phenomenon. We also established that the bioflavonoid at high concentrations (3 and 5 mM) in combination with histamine (0.01 μM) slowed down the generation of superoxide anion compared to the independent action of histamine (**Fig. 1**). M. Jafarinia *et al.* state that quercetin has the potential to reduce the most significant pathologies of asthma such as eosinophil and neutrophil recruitment, the activation of bronchial epithelial cells, collagen and mucus production and airway hyperactivity (Jafarinia *et al.,* 2020). It is likely that higher concentrations of quercetin are embedded in the membrane of cells (Arora *et al.,* 2000), in particular blood neutrophils, and this leads to a violation of the binding of histamine receptors to histamine and to a decrease in the formation and release of superoxide anion by these cells into the blood plasma. It is known that histamine modulates the release of free radicals by neutrophils (Sklyarov O., 2017).

Therefore, histamine, quercetin, and their combination led to the intensification of superoxide anion generation in the blood plasma of rats. Quercetin is known as an antioxidant, but the results of our studies show the opposite effect. The study of the effect of quercetin on the indicators of the body's protective potential under the increased content of fructose in the diet of rats showed that there was no significant effect of the bioflavonoid on the protective potential of animals at the initial stage of obesity.

O. I. Bishko *et al*. established that exogenous histamine administration in rats at doses of 1 and 8 μg/kg increases the superoxide dismutase activity in rats' blood plasma. The dose of 1 μg/kg corresponds to a histamine concentration of 1.6 μM, and 8 μg/kg – 12.8 μM. Superoxide dismutase is an enzyme that neutralizes superoxide anions. Under the action of histamine at a dose of 1 μg/kg on the 1st day of the experiment, there was an increase in the activity of glutathione peroxidase, which neutralizes both hydrogen peroxide and lipid hydroperoxides. However, on the 7th day of action, an increase in the activities of both glutathione peroxidase and catalase was noted (Bishko *et al*., 2014). Thus, our *in vitro* studies confirmed the increase in the content of superoxide anion by histamine in the blood plasma.

Effect of histamine and quercetin on ATP content in blood plasma of rats. An increased content of ATP in the blood plasma of rats was observed under quercetin

effect (**Fig. 2**). Quercetin at concentrations 0.1 and 0.5 mM increased ATP content by 109 and 72%, respectively. A more intense increase in the content of the indicator occurs under the influence of quercetin in the concentrations: 0.3; 1; 3; 5 mM (by 402; 399; 644; 307%, respectively). Quercetin is a modulator of enzyme activity; it inhibits enzymes with ATP-binding sites. In particular, protein kinases, mitochondrial ATPases, myosin, Na^+/K^+ and Ca^{2+} plasma ATPases, topoisomerase II, ATP-dependent transport P-glycoproteins (P-gp – carriers with high cellular permeability – P-permeability), and P-glycoproteins of the MRP1 subgroup and 2 (multidrug-resistant proteins). The selective attachment of quercetin to the active ATP-binding center of the enzyme probably caused the inhibition of the enzyme. (Slesarchuk, 2014). We presume that the increased content of ATP in the blood plasma of rats under the action of quercetin was due to the disruption of the work of ATP-dependent enzymes and the release of ATP outside the blood cells. Quercetin, despite its plant origin, is a foreign compound for the body. Therefore, it is neutralized by glutathione transferase and cytochrome *p* 450, which can lead to the formation of even more reactive compounds compared to the original form. As a result, all this leads to varying degrees of influence (not dose-dependent) of quercetin or its metabolites on the work of enzymes, ATP-ases and, accordingly, different changes in the content of ATP in the blood plasma of rats. Scientific literature shows that adding quercetin at a concentration of 5–25 μM to blood stabilizes the erythrocyte membrane (Ferrali *et al.,* 1997). However, in our studies, quercetin in higher concentrations (0.1–5 mM) probably causes damage to erythrocyte membranes by embedding quercetin in the membrane and increasing the peroxidic oxidation of lipids in it, and the release of ATP into the blood plasma.

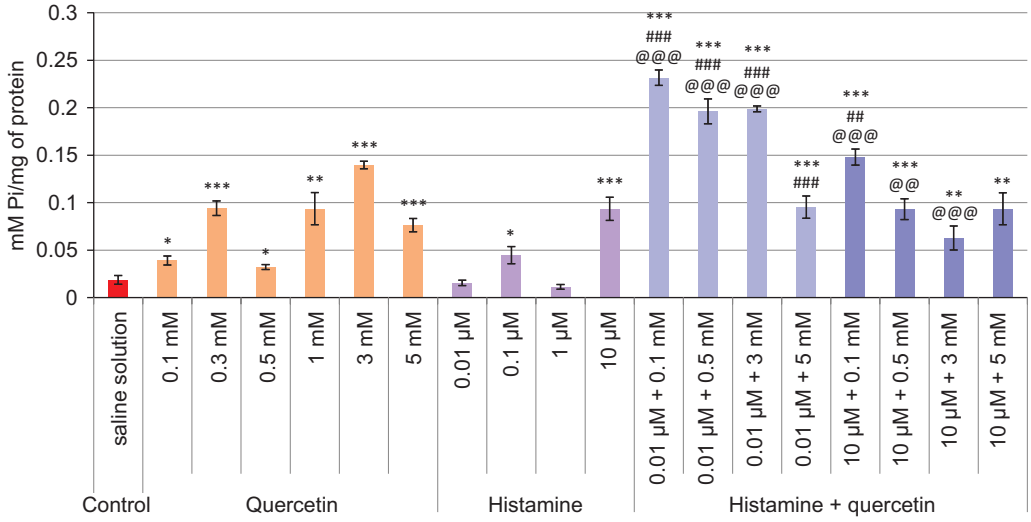

Fig. 2. The content of ATP in blood plasma of rats under the action of quercetin, histamine and the combination of histamine and quercetin (* – p ≥0.95; ** – p ≥0.99; *** – p ≥0.999; # – compared to the effect of histamine of the corresponding concentration; @ – compared to the effect of quercetin of the corresponding concentration)

Histamine was added to whole blood in concentrations of 0.1 and 10 μM, and an increase in the content of the studied substance was found in the plasma by 139 and

398%, respectively. There are no significant differences between the effects of either of the concentrations of histamine (0.01 and 1 μM) on ATP content compared with control (**Fig. 2**). Blood basophils and mast cells are known to release kinins, histamine, leukotrienes, prostaglandins, serotonin, and ATP under the action of external factors, including histamine (Bishko, 2012).

Simultaneous action of histamine and quercetin has been shown to lead to a significant increase in the ATP content in the plasma. The effect of histamine at a concentration of 0.01 μM with simultaneous exposure to quercetin leads to a more pronounced increase in ATP content compared with the histamine at a concentration of 10 μM. (**Fig. 2**). Therefore, the combined action of quercetin and histamine does not return the ATP content to normal. ATP is known as a regulator of cell functions through the reception of purinergic receptors, which are present in many cells, including macrophages, platelets, and lymphocytes. (Wang, 2005). Therefore, changes in ATP content during the combined administration of histamine and quercetin in the blood are a negative phenomenon. The high content of ATP in the blood plasma will produce changes in the functional processes in both blood cells and other cells of the body (for example, vascular smooth muscles).

Thus, quercetin causes an increase in the ATP content in the blood plasma of rats. Histamine causes the same effect only at concentrations of 0.1 and 10 μM. The combined introduction of histamine and quercetin into the blood also increased the ATP content.

A two-way ANOVA analysis of the effects of histamine and quercetin on the content of superoxide anion and ATP in the blood plasma of rats. A two-way ANOVA test showed a significant interaction between the effects of histamine and quercetin on the content of superoxide anion and ATP in the blood plasma of rats. Thus, the share of influence of histamine and quercetin on the content of superoxide anion is 23% and 19%, respectively (**Fig. 3**). The shares of the influence of both histamine and quercetin on ATP content are equal – 27% (**Fig. 3**).

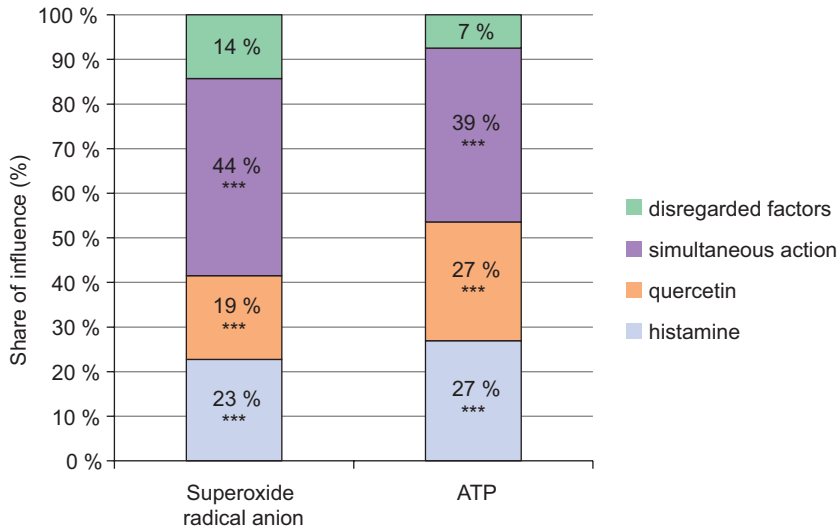

Fig. 3. The results of Anova two-factor analysis of the parameters of the content of superoxide anion and ATP in blood plasma of rats under the action of quercetin, histamine and the combination of histamine and quercetin (*** – p ≥0.999)

A significant part of the impact is the combined effect of histamine and quercetin on the values of the studied indicators. We have established that the share of such impact on the content of superoxide anion is 44%, and on the content of ATP – 39% (**Fig. 3**). Notice that all the results of ANOVA analysis are highly reliable, and the share of influence of unaccounted factors is low (**Fig. 3**).

The ANOVA analysis showed that histamine and quercetin have the same effect on releasing superoxide anion and ATP from blood cells into plasma, and their combined action only strengthens this effect.

CONCLUSION

1. Histamine and quercetin added to the blood of rats led to a significant increase in the content of superoxide anion in the plasma. Quercetin induced an increase in the amount of the studied product in a dose-dependent manner.
2. Quercetin at the minimum and therapeutical (3 mM) concentrations reduced the generation of superoxide anions in combination with the action of histamine in the maximum concentration. The bioflavonoid at a low dosage enhances and at a high dosage slows down the accumulation of superoxide anion in combination with the action of histamine at a minimum concentration compared to samples to which histamine was added at the specified concentration.
3. Histamine in concentrations of 0.1 and 10 μM causes an increase in ATP content in the blood plasma of rats. Quercetin, as well as the combined addition of histamine and quercetin to the blood, leads to a significant increase in ATP content.
4. Histamine and quercetin have the same effect on the amount of superoxide anion and ATP. The simultaneous addition of biogenic amine and quercetin to blood samples has a significant effect on the content of superoxide anion and ATP in plasma.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights: This article does not contain any studies with human subjects performed by the any of the authors.

Animal studies: All international, national and institutional guidelines for the care and use of laboratory animals were followed.

AUTHOR CONTRIBUTIONS

Conceptualization, [H.N.; G.V.; B.N.; Z.A.; M.S.; H.A.]; methodology, [H.N.; G.V.; B.N.; Z.A.; M.S.; H.A.]; validation, [H.N.; G.V.; B.N.; Z.A.; M.S.; H.A.]; formal analysis, [H.N.; G.V.; B.N.; Z.A.; M.S.; H.A.]; investigation, [H.N.; G.V.; B.N.; Z.A.; M.S.; H.A.B.]; resources, [H.N.; G.V.; B.N.; Z.A.; M.S.; H.A.]; data curation, [H.N.; G.V.; B.N..; Z.A.; M.S.; H.A.]; writing – original draft preparation, [H.N.; G.V.; B.N.; Z.A.; M.S.; H.A.]; writing – review and editing, [H.N.; G.V.; B.N.O.; Z.A.; M.S.; H.A.]; visualization, [H.N.; G.V.; B.N.; Z.A.; M.S.; H.A.]; supervision, [H.N.; G.V.; B.N.; Z.A.; M.S.; H.A.]; project administration, [H.N.; G.V.; B.N.; Z.A.; M.S.; H.A.]; funding acquisition, [-].

All authors have read and agreed to the published version of the manuscript.

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ВМІСТ СУПЕРОКСИД-АНІОН РАДИКАЛА Й АТФ ПЛАЗМІ КРОВІ ЩУРІВ ЗА ДІЇ ГІСТАМІНУ І КВЕРЦЕТИНУ

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Вступ. Гістамін є одним із універсальних біогенних амінів, який бере участь у імунній відповіді й алергічних розладах. Гістамін і АТФ можуть діяти як ліганди в організмі. У плазмі АТФ є потужним вазодилататором, який стимулює утворення NO та простагландинів і, що дуже важливо, може компенсувати місцеву симпатичну вазоконстрикцію. Аденозинтрифосфат вивільняється в плазму з еритроцитів і ендотеліальних клітин, і концентрація в плазмі збільшується як в артеріях, так і у

венах, що живлять скелетні м'язи, які скорочуються. Беручи це до уваги, актуальним є вивчення впливу гістаміну в поєднанні з кверцетином, який пригнічує вивільнення гістаміну з клітинних депо, на вміст супероксид-аніона й АТФ у плазмі крові.

Матеріали та методи. У дослідженнях використовували нелінійних білих щурів-самців. До цільної крові додавали кверцетин, щоб кінцева концентрація становила 0,1; 0,3; 0,5; 1; 3; 5 мМ. В інших експериментах до крові додавали розчин гістаміну до кінцевої концентрації 0,01; 0,1; 1; 10 мкМ. У серії дослідів до крові додавали гістамін (0,01 і 10 мкМ) і кверцетин (0,1; 0,5; 3; 5 мМ) у різних можливих комбінаціях. В експериментах використовували плазму крові. Кров, до якої додавали фізіологічний розчин, використовували як контроль. У відібраних зразках визначали вміст супероксид-аніона і аденозинтрифосфату.

Результати. Гістамін, кверцетин, а також їхнє поєднана дія призводять до інтенсифікації утворення супероксид-аніона у плазмі крові щурів. Відомо, що кверцетин є антиоксидантом, проте нашими дослідженнями встановлено протилежний ефект. Кверцетин спричиняє підвищення вмісту АТФ у плазмі крові щурів. Гістамін зумовлює такий же ефект у концентраціях 0,1 і 10 мкМ. Спільне введення до крові гістаміну і кверцетину також сприяє підвищенню вмісту АТФ. Результати дисперсійного аналізу доводять, що і гістамін, і кверцетин однаково впливають на вивільнення супероксид-аніона й АТФ з клітин крові у плазму. А їхня сукупна дія лише посилює ефект вивільнення досліджуваних продуктів.

Висновки. Кверцетин, гістамін і їхня поєднана дія активізують утворення супероксид-аніона й АТФ у плазмі крові щурів.

Ключові слова: кверцетин, гістамін, супероксид-аніон, АТФ

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