References

1.Markov II, Petrov ES, Markova VI. Universalnyy metod elektivnogo vyyavleniya argirofilnykh struktur. Morfologicheskie vedomosti. 2016;1:116–119. [in Russian]
2. Nozdrachev AD. Vegetativnaya reflektornaya duga. Sankt-Peterburg: Nauka, 2018:232. [in Russian]
3. Sotnikov OS. Dinamika struktury zhivogo neyrona. Leningrad:, Nauka, 2013: 159. [in Russian]
4. Sotnikov OS, Markov II. Kontseptsiya retikulyarnoy organizatsii nervnoy tkani Aleksandra Dogelya. Morfologicheskie vedomosti. 2018;1:8–19. [in Russian]
5, Chuchkov VM, Gelashvili PA., Nikolenko VN, Sabel'nikov NE., Koshev VI, Gelashvili OA, et al. Strukturnye osnovy innervatsii i vaskulyarizatsii skeletnykh myshts i myshechnoy obolochki polykh organov mlekopitayushchikh i cheloveka. Samara: OOO «Ofort» Samara, 2010:203 s. [in Russian]
6. Shvalev VN. Mirovoe znachenie kazanskoy neyrogistologicheskoy shkoly. Zaslugi ee rukovoditelya professora A.N. Mislavskogo i sokhranenie ee traditsiy v posleduyushchikh issledovaniyakh. Kazanskiy meditsinskiy zhurnal. 2013; 94(3): 424–429. [in Russian]
7. Altschuler S, Escardo J, Lynn R. The central organization of the vagus nerve innervating the large intestine of the rat. J. Gastroenterology, 2013;104:502 – 509 doi: 10.1016/0016–5085(93)90419–d
8. Bullock TH. Have brain dynamics evolved? Should we look for unique dynamics in the sapient species? Neural Comput. 2013;15(9):2013–27. doi: 10.1162/089976603322297269.
9. Costa M, Wiklendt L, Keightley L, Brookes SJH, Dinning PG, Spencer NJ. New insights into neurogenic cyclic motor activity in the isolated guinea – pig large intestine. Neurogastroenterol Motil. 2017;29(10):1–13. doi: 10.1111/nmo.13092
10. Tai C, Booth A, de Groat C. Bladder and urethral sphincter responses evoked by microstimulation of S2 sacral spinal cord in spinal cord intact and chronic spinal cord injured cats. Exp. Neurol., 2014;190(l5):171–183.

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THE EFFECT OF CARBON MONOXIDE'S DONOR CORM-2 ON ERYTHROCYTE AQUAPORINS

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Carbon monoxide is part of group of gas transmitters, which are produced by almost all cells and involved in many mechanisms of internal and external communication between cells. Carbon monoxide-releasing molecules are promising for therapeutic use. Such donors include tricarbonyldichlororuthenium (II) dimer. For the research, the aquaporins 1 and 3 were blocked in erythrocyte membranes with the use of mercury chloride and silver nitrate, and then they were added to hypo–, iso– and hyperosmolar solutions. However, tricarbonyldichlororuthenium (II) dimer does not bind to aquaporins 1, but this effect is due to the effect on aquaporins 3. This effect is dose-dependent: the most active concentration was 6–50 μM. It is arguable that tricarbonyldichlororuthenium (II) dimer increases the resistance of erythrocytes to hypertensive stress, stabilizes their volume in a hyper- and hypo-tonic environment by activating aquaporins 3.

Key words: AQP1, AQP3, gas transmitter, erythrocyte membrane.

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ВПЛИВ ДОНОРА МОНООКСИДУ КАРБОНУ CORM-2 НА АКВАПОРИНИ ЕРИТРОЦИТІВ

Монооксид карбону, який належить до групи газотрансміттерів, продукується практично всіма клітинами організму і бере участь у багатьох механізмах міжклітинної сигналізації. Сполуки-донори монооксиду карбону є перспективними для терапевтичного застосування. До таких донорів належить димер трикарбонілдихлororутенію (II). Для дослідження реакції аквапорінів 1 і 3 проводили їхнє блокування в мембранах еритроцитів з використанням хлориду ртуті та аргентум нітрату й отриману суспензію поміщали у гіпо–, ізо– і гіперосмолярні розчини. Було встановлено, що димер трикарбонілдихлororутенію (II) впливає на транспорт води в еритроцитах шляхом дії на аквапорини 3. Цей ефект дозо-залежний: найбільш активна концентрація становила 6–50 μМ. Отже, димер трикарбонілдихлororутенію (II) підвищує стійкість еритроцитів до гіпертонічного стресу, стабілізує їх об'єм в гіпер- і гіпотонічному середовищі впливаючи на аквапорини 3.

Ключові слова: AQP1, AQP3, газотрансміттер, мембрана еритроциту.

This work is a fragment of the research project "The effect of certain vasoactive substances on the central and peripheral lymphoid organs of white mice", state registration No 0117U001764.

Since 1993, carbon monoxide (CO) has been regarded not only as a toxic gas. It is also being studied as a substance that plays an important physiological role in the body [7]. Prior to that, it was found that CO is constantly produced in the human body and significantly increases under conditions accompanied by abnormal decomposition of red blood cells (RBC) [4]. Over time it was found that CO is freely diffusible and traverses all membranes, bypassing transporters and thus can rapidly mediate

functional changes in the cell. Proximal targets for CO would include those on or near the cellular surface, such as soluble guanylyl cyclase, heme-containing potassium channels, caveolar NO-synthase and surface NADPH oxidase [12].

At physiological concentrations, CO has been shown to mediate signaling processes in the brain, liver and endothelium. Besides, CO regulates vascular tone and the production of inflammatory mediators, and also it is implicated in a number of mechanisms mitigating apoptosis and ischemic injury [7].

Promising for therapeutic use, the inhalation of gaseous CO is very difficult. It is necessary to accurately dose it, which is very difficult to do with a gaseous form. For this purpose, carbon monoxide donors (CORMs) were synthesized. These substances make it possible to deliver CO as intended and strictly control the amount of CO released in tissues and cells [9]. Tricarbonyldichlororuthenium (II) dimer (CORM–2): $[Ru(CO)_3Cl_2]_2$ is one of such substances. CORM–2 also showed an anti-inflammatory effect, it suppresses the lipopolysaccharide-induced airways, intensifies plasmatic coagulation and attenuates fibrinolysis *in vitro* in plasma [4].

The RBCs are important model for studying the effect of various biologically active substances on aquaporins (AQPs). The AQPs are expressed in the cell plasma membranes of various epithelial, endothelial and other cell types. AQP1 is an efficient water transporting protein that does not transport H^+, urea or other small solutes. Defects of aquaporin channels lead to evident defects in urinary concentrating ability and tumor angiogenesis, as well as reduced secretion of cerebrospinal fluid by the choroid plexus and of aqueous fluid by the ciliary epithelium [1]. Sulfhydryl-reactive mercurials are the best established water channel inhibitors. The mercurials inhibit AQP1 water permeability by covalent modification of cysteine–187 in the AQP1 sequence [3].

Considering all of the above, it is necessary to emphasize the importance of research on the effect of carbon monoxide and CORMs on cells and their membranes, especially blood. There is no accurate data on the effect of CORM–2 on the parameters of RBCs volume changes; its effect on aquaporins (AQP1 and AQP3) is absent.

The purpose of the work was to study the effect of carbon monoxide from tricarbonyldichlororuthenium (II) dimer on erythrocyte aquaporins.

Materials and methods. Human venous blood was obtained from 30 donors. Blood was collected into Vacutainers coated with sodium heparin (25 IU/ml), (Greiner, Kremsmunster, Austria). The samples were centrifuged (320 g for 15 min, 21°C); the platelet-rich plasma and the white blood cells coat were removed. The erythrocyte sediment was washed twice with three parts of an iso-osmotic NaCl solution (150 mM) containing 5 mM Na-phosphate buffer (pH 7.4) under the same centrifugation conditions. Last time, the erythrocytes were washed with medium (containing 150 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, 10 mM glucose), under the same centrifugation conditions. After that, packed RBCs were transferred to ice and stored for no longer than 12 hours.

The washed RBCs were divided into 3 groups. The first group was control, the second was for pre–incubation with an aquaporin 1 blocker ($HgCl_2$, 0.2 μM; Sigma Aldrich, Steinheim, Germany), and the third – for pre-incubation with an aquaporin 3 blocker ($AgNO_3$, 5 μM; Sigma Aldrich, Steinheim, Germany). After 10 minutes of incubation of RBCs (Hb: 0.2 g/dl) with aquaporin blockers, they (0.350 ml aliquots) were placed in a medium (volume 3.150 ml) of various osmolarity:

Protocol 1: erythrocytes in isotonic incubation solution. The erythrocytes were suspended in iso-osmotic (320 mOsm) saline solution, containing (in mM): NaCl 150, KCl 1.0, MgCl2 1.0, glucose 10.

Protocol 2: shrinkage of red blood cells. Hypertonic (420 and 520 mOsm) salt solution (SS), containing (in mM): NaCl 150, KCl 1.0, MgCl2 1.0, $C_{12}H_{22}O_{11}$ 100 (420 mOsm) or 200 (520 mOsm), glucose 10. Protocol 3: swelling of red blood cells. Hypotonic (220 mOsm) SS, containing (in mM) NaCl 100, KCl 1.0, MgCl2 1.0, glucose 10.

Aliquots of the suspension with RBCs were poured into quartz cuvettes and placed in the wells of a photometer. Light scattering of samples to light with 800 nm wave length, relative to the value passing through the suspension of quiescent RBCs incubated in different solutions, was measured at 21C in the photometer (Shimadzu UV–2600, Japan).

To determine the effect of CO on the activity of AQP1 and AQP3, a freshly prepared solution was added to the resulting solution CORM–2 (tricarbonyldichlororuthenium (II) dimer, 6, 10, 50, 100 и 200 μM; Sigma Aldrich, Saaint Lois, USA), and dissolved in DMSO (in the final solution, the concentration of DMSO did not exceed 0.1 %). The concentration of CORM-2 was 200, 100, 50, 25, and 6 μM. After 5 minutes, measurements were made on the spectrophotometer. For the control, inactivated CORM-2 (iCORM-2), which has lost CO, was added to the erythrocyte suspension. For the visual control of changes, the erythrocytes were photographed using the microscope.

All data of absorbance measurements are presented as means ± standard error of the mean. Since average slopes under a given condition did not differ significantly between those individual donors at our disposal, it was possible to combine maximal-slope results obtained from RBCs of different donors. Statistical analysis of slope data was performed by one-way analysis of variance (ANOVA) for repeated measures, and P-values corrected for multiple comparisons by the Bonferroni-Holm procedure <0.05, were taken as statistically significant.

In this study, the code of ethics established by the Helsinki Declarations of 1964 and revised in 2000, was followed. The study protocol was reviewed and approved by the Kherson State University Ethical Committee (ethical committee no: 2020/6).

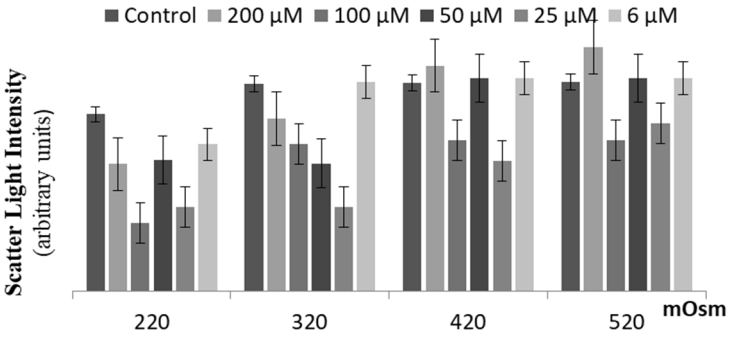

Fig. 1. Comparative characteristics of the light scattering index of intact and CORM-2 erythrocytes exposed to varying osmolarity of the incubation medium. Statistically significant differences of each group from the control at p≤0.05

Results of the study and their discussion. The effect of CORM-2 on the volume of red blood cells in solutions of different osmotic concentrations. In this experiment, the red blood cells changed their ability to withstand the osmotic shock after the addition of CORM-2 to the suspension of RBCs. In the hypo-osmotic medium, the erythrocytes incubated with CORM-2 (at the concentration of 200 μM) swelled more, compared with intact ones (cell volume increased by 5±0.2 %). Also, CORM-2 at this concentration, in the isosmotic medium, caused RBCs to swell, their volume increased by 3±0.1 %. In the hyperosmotic environment (420 and 520 mOsm), RBCs treated with CORM-2, on the contrary, were of a smaller volume than intact cells (by 2±0.1 % and 3±0.1 %, respectively) (fig. 1).

After the addition of CORM-2 at the concentration of 100 μM to the RBCs suspension, we got different effect: the erythrocytes did not swell in the hypo-osmotic medium (as opposed to intact ones), their volume was the same as that of erythrocytes in the isosmotic medium. In the hyperosmotic medium (420 and 520 mOsm), the addition of CORM-2 prevented the compression of RBCs only by 2±0.1 %.

The addition of CORM–2 (at the concentration of 50 μM) led to the fact that the volume of RBCs increased in the hypo-osmotic and isotonic medium (by 2±0.1 % and 3±0.2 %, respectively). At the same time, no changes occurred in the hyperosmotic environment.

At the concentration of 25 μM, CORM-2 affected the RBCs the same way under different conditions: in hypotonic, isotonic and hypertonic solutions, and their volume increased. In the hypo-osmotic and isotonic mediums, it increased by 2±0.1 %, in the hyperosmotic (420 and 520 mOsm) medium – by 4±0.2 %. At the concentration of 6 μM, CORM–2 influenced the RBCs only in the hypo-osmotic environment – the cell volume increased by 2±0.1 %. In other cases, CORM-2 did not significantly affect the RBCs at this concentration (fig. 2).

The effect of CO from CORM-2 on erythrocytes under the conditions of AQP1 blockade. In order to find out the effect of CO from CORM-2 on AQP1, we previously incubated the studied RBCs with $HgCl_2$ at the concentration of 0.2 μmol. After 10 minutes, we performed all the manipulations, as in the previous experiment. $HgCl_2$ was used as positive control for inhibition.

In order to find out what affects AQP1: either CO which was released from CORM-2 or ruthenium chloride (which remained after CO removal), we have also added inactivated CORM-2 (iCORM-2) to the erythrocyte suspension. After the incubation of RBCs with the blocker AQP1, they swelled at different concentrations of iCORM-2 and CORM-2 in the medium with different osmolarity (220, 320, 420 and 520 mOsm). In this case, CORM-2 led to more significant swelling of the RBCs than in case of iCORM-2 (tab. 1).

Table 1

The effect of CORM-2 and iCORM-2 on the scatter light intensity of the erythrocyte suspension under the conditions of varying osmolarity of the incubation medium (the mean value of concentrations 200, 100, 50, 25 and 6 μM), M±m

Osmolarity of the incubation medium	Control	Inactivated CORM-2 (without CO)	CORM-2 (with CO)
220 (hypo-osmotic)	2.738±0.1	2.689±0.02*	2.638±0.1*
320 (iso-osmotic)	2.835±0.1	2.775±0.03*	2.721±0.03*
420 (hyperosmotic)	2.893±0.05	2.825±0.02*	2.756±0.1*
520 (hyperosmotic)	2.881±0.06	2.835±0.5*	2.765±0.1*

Note:* – significant difference from the control group (P≤0.05)

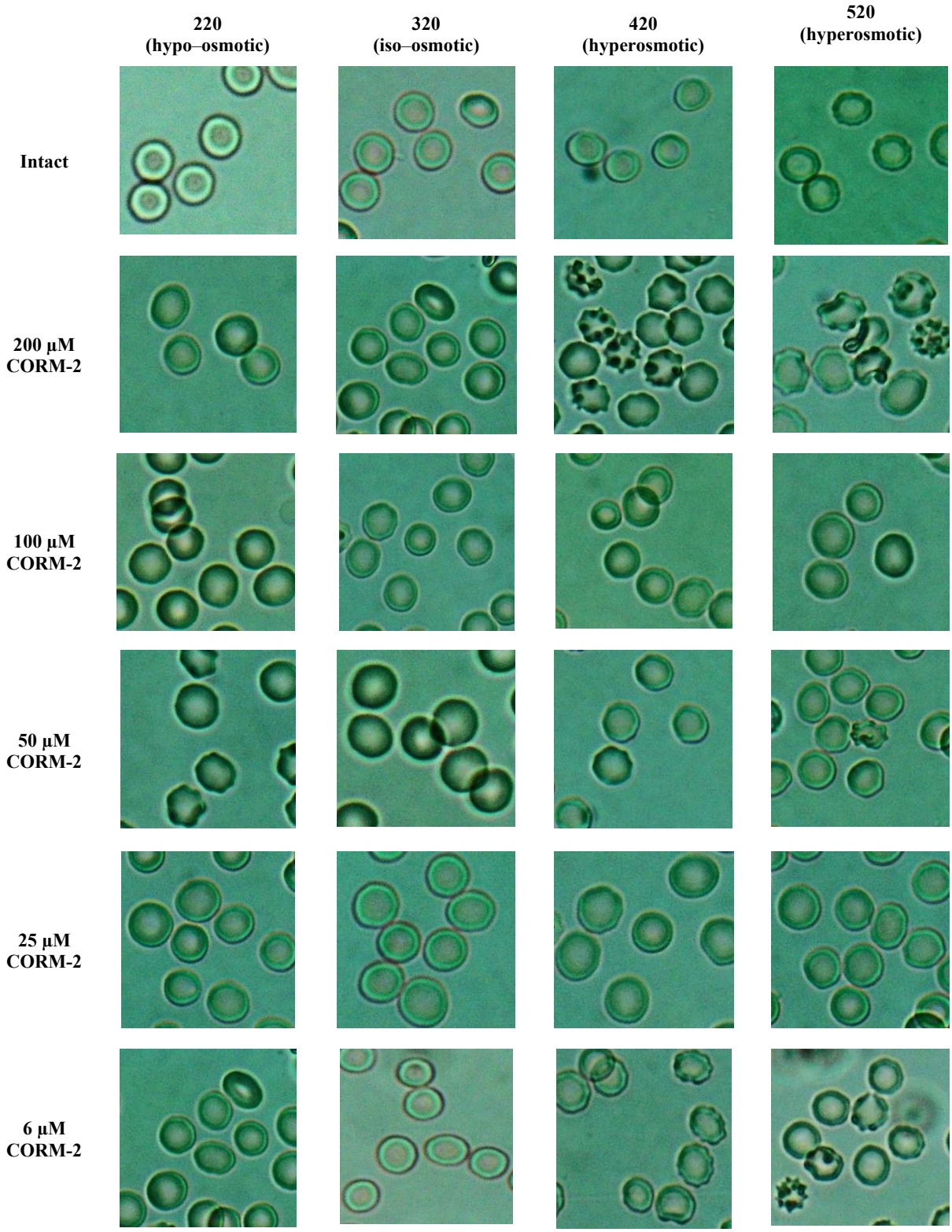

Fig. 2. Comparative micrographs of red blood cells under the influence of CORM-2 in different concentrations (× 100)

In a hypo-osmotic medium (220 mOsm), the light scattering index (absorbance) decreased from 2.735±0.14 to 2.692±0.134 (after addition of iCORM-2) and 2.647±0.079 (after addition of CORM-2). Iso-osmotic (320 mOsm): from 2.831±0.084 to 2.775±0.138 (iCORM-2) and 2.713±0.081 (CORM-2). It was also after the incubation in hypertonic media. In the environment with 420 mOsm: from 2.882±0.144 to 2.821±0.084 (iCORM-2) and 2.764±0.082 (CORM-2). In the medium of 520 mOsm, the light scattering index decreased from 2.889±0.087 to 2.832±0.057 (iCORM-2) and 2.767±0.083 (CORM-2). Thus, the preliminary blocking of AQP1 showed that the mechanism of influence of CO from CORM-2 on the erythrocyte volume is not associated with this channel.

The effect of CORM-2 on red blood cells in the conditions of the blockade of AQP3. Aquaporin 3, or AQP3, or aquaglyceroporin, has only relative specificity for water and also gates other low molecular weight uncharged compounds such as urea and glycerin.

The results of the study of the effect of CORM-2 on the RBCs volume after AQP3 blockade were very interesting. The dose-dependent effect of CORM-2 on AQP3 in membranes of the RBCs has been observed. After CORM-2 was added at the concentration of 6, 25, and 50 μM in the hypertonic solution (520 mOsm), no changes occurred (fig. 3 a, b, c). The preliminary blockade of AQP3 and the absence of the effect of changes in the volume of the RBCs confirm the hypothesis that CORM-2 is related to this type of aquaporins.

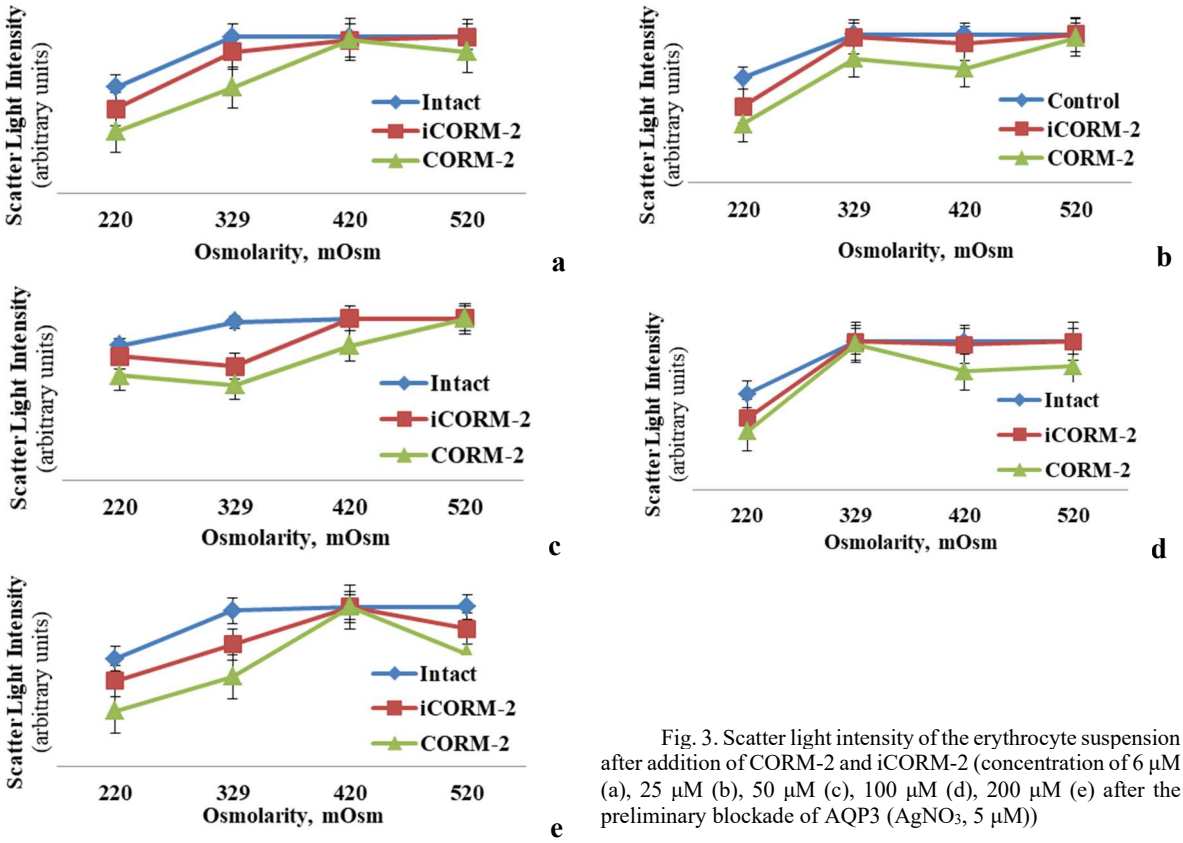

Fig. 3. Scatter light intensity of the erythrocyte suspension after addition of CORM-2 and iCORM-2 (concentration of 6 μM (a), 25 μM (b), 50 μM (c), 100 μM (d), 200 μM (e) after the preliminary blockade of AQP3 ($AgNO_3$, 5 μM))

At the concentration of 100 μM, the volume of erythrocytes did not change only in the iso-osmotic medium (fig. 3 d). At the concentration of 200 μM, the erythrocyte volume did not change only in the hypertonic medium of 420 mOsm (fig. 3 e). Similar results were registered under the conditions of addition of iCORM-2 at the same concentrations. In all other cases, the increase in the volume of RBCs was observed. Only after CORM-2 was added at the concentration of 6 μM, the volume of RBCs did not change in both hypertonic solutions. The results have confirmed the assumption about the dose-dependent effect of CORM-2 on erythrocyte aquaporins.

The group of gas transmitters continues to grow, and currently includes, in addition to nitrogen monoxide, hydrogen sulfide and carbon monoxide [7]. Gas mediators are produced almost entirely by cells, and due to their high lipophilicity, they are involved in many mechanisms of intracellular and intercellular communication [6].

Significant progress in the studies of reactions mediated by gas transmitters is achieved in connection with the discovery of the ability of certain chemical compounds to reproduce the effects of these signaling molecules, which are valid [2, 7]. CORM-2 carbon monoxide donor is the example of such a substance.

In adult organism, CO is produced endogenously in the amount of 20 μM * h^{-1} [8]. At the same time, CO production increases with various diseases [5]. It has been proved that CO or CORM-2, in low concentrations, stimulates angiogenesis and has anti-ischemic and anti-apoptotic effect [11]. The use of carbon monoxide donors involves direct introduction of CORM-2 into the vascular bed.

To date, it was unknown how CORM-2 affects the RBCs. In this study, it has been found that CORM-2 (CO released from it) dose-dependently (like other gas transmitters) affects the functional properties of the erythrocyte membrane. This is expressed in the change in the volume of RBC in hypo-, iso- and hypertonic solutions. After addition of 200 μM CORM-2 to the erythrocyte suspension, their volume in the hypo-osmotic and iso-osmotic mediums increased.

In the hyperosmotic medium, the processed CORM-2 erythrocytes, on the contrary, were compressed. Processing of the RBCs by CORM-2 at the concentration of 100 μM protected them from swelling in the hypo-osmotic medium, their volume was the same as that of RBCs in the isosmotic medium. In the hyperosmotic medium, the addition of CORM-2 reduced erythrocyte compression. After the addition of CORM-2 at the concentration of 50 μM, the volume of the RBCs increased in the hypo-osmotic and isotonic mediums, but in the hyperosmotic medium, no changes occurred.

However, we observed the increase in the RBCs volume in hypotonic, isotonic and hypertonic after the addition of CORM-2 at the concentration of 25 μM. At the concentration of 6 μM, CORM-2 affected on the RBCs only in the hypo-osmotic environment – there was the increase in volume. In other cases, CORM–2 at this concentration did not significantly affect the RBCs. Thus, it can be suggested that CO, which is released from CORM-2, affects special protein channels that transport water to the cell – the aquaporins. It is interesting that similar processes occur in mitochondria: a publication [10] indicates that CO triggered mitochondrial swelling.

The erythrocyte membrane has specialized channels for passive oxygen transport. These channels have been identified as aquaporins (AQP). It is believed that aquaporins are proteins of water channels. But to date, aquaporins that transport CO, CO_2, O_2, and NH_4 have also been identified [1]. Two types of aquaporins are known in the RBCs: AQP1 and AQP3. It is known that AQP1 provides high permeability of the erythrocyte membrane to water, AQP3 – to water, glycerin and urea [9]. Mercury chloride $HgCl_2$ is recognized hAQP1 inhibitor in human erythrocytes [1]. There are a lot of aquaporins in erythrocyte membranes (approximately $2 * 10^5$ molecules/cell). Recent studies have shown that water channels, in particular, aquaporin AQP1, participate in the transfer of oxygen and carbon dioxide through the erythrocyte membrane [9]. This study found out how CORM–2 affects these types of aquaporins. The blockade of AQP1 using $HgCl_2$, and AQP3 using $AgNO_3$ made it possible to determine the effect of CO from CORM–2 on the RBCs.

After incubated with the AQP1 blocker, iCORM–2 and CORM–2 led to the swelling of the RBCs at different concentrations in the medium with different osmolarity. At the same time, the addition of iCORM-2 and CORM-2 contributed to the increase in the volume of RBCs. Freshly prepared CORM–2 led to RBCs swelling the most. The results indicate that the effect of CORM-2 on RBCs volume is not due to its binding to AQP1. Thus, we suggested that another, no less important channel that can interoperate with CORM-2, is AQP3.

AQP3 (or aquaglyceroporin), has only relative specificity for water. Following blockade of AQP3, the dose-dependent effect of CORM-2 on AQP3 of the RBCs has been observed. After the addition of CORM-2 to insignificant concentration (6, 25, 50 μM) in the hypertonic solution (520 mOsm), no changes occurred. The preliminary blockade of AQP3 and the absence of the effect of changes in the volume of RBCs confirm the hypothesis that CORM-2 is related to this group of aquaporins. iCORM-2 also demonstrates its activity against AQP3. Thus, the assumption is confirmed [6] that in addition to the released CO from CORM-2, erythrocytes are also affected by iCORM-2, which is formed after the release of CO.

Conclusion

This study has shown that CORM-2 has an effect on the transport of water in the RBCs. However, CORM-2 does not bind to AQP1. This effect is due to the effect on AQP3. This effect is dose-dependent: the most active concentration was 6-50 μM. It can be argued that CORM-2 increases the resistance of RBCs to hypertensive stress, stabilizes the volume of erythrocytes in a hyper– and hypotonic environment, by activating AQP3. This erythrocyte aquaporin is convenient model for further research, as it plays an important role in skin hydration, wound healing and tumor growth. It is also proved that aside from CO, which is released after addition CORM-2, ruthenium which previously formed carbonyl compound acts on the erythrocyte membrane.

References

1. Abir-Awan M, Kitchen P, Salman MM, Conner MT, Conner AC, Conner AC, Bill RM. Inhibitors of mammalian aquaporin water channels. Int J Mol Sci. 2019; 20(7): 1589. https://doi.org/10.3390/ijms20071589.

2. Beschasnyi S, Hasiuk O. CO-releasing molecule (CORM-2) in the regulation of Ca^{2+}–dependent K^+–permeability of erythrocyte. Ukr. ž. med. bìol. sportu. 2020; 5(2), 166–171. https://doi.org/10.26693/jmbs05.02.166.

3. Huisjes R, Bogdanova A, van Solinge WW, Schiffelers RM, Kaestner L, van Wijk R. Squeezing for life – properties of red blood cell deformability. *Front Physiol.* 2018; 9: 656. https://doi.org/10.3389/fphys.2018.00656.

4. Johnson TE, Wells RJ, Bell A, Nielsen VG, Olver CS. Carbon monoxide releasing molecule enhances coagulation and decreases fibrinolysis in canine plasma exposed to *Crotalus viridis* venom *in vitro* and *in vivo*. Basic Clin Pharmacol Toxicol. 2019; 125(4): 328–336. doi: 10.1111/bcpt.13242.

5. Lin CC, Hsiao LD, Cho RL, Yang CM. Carbon monoxide releasing molecule-2-upregulated ROS-dependent heme oxygenase-1 axis suppresses lipopolysaccharide-induced airway inflammation. Int J Mol Sci. 2019; 20(13): 3157. https://doi.org/10.3390/ijms20133157.

6. Liu Y, Wang X, Xu X, Qin W, Sun B. Protective effects of carbon monoxide releasing molecule-2 on pancreatic function in septic mice. Mol Med Rep. 2019; 19 (5): 3449–3458. https://doi.org/10.3892/mmr.2019.10049.

7. Motterlini R, Foresti R. Biological signaling by carbon monoxide and carbon monoxide-releasing molecules. Am J Physiol Cell Physiol. 2017; 312(3): 302–313. https://doi.org/10.1152/ajpcell.00360.2016.
8. Queiroga CS, Almeida AS, Alves PM, Brenner C, Vieira HL Carbon monoxide prevents hepatic mitochondrial membrane permeabilization. BMC Cell Biol. 2011; 12: 1–8. https://doi.org/10.1186/1471-2121-12-10.
9. Qureshi OS, Zeb A, Akram M, Myung-Sic K, Kang J-H, Kim HS, Majid A, Han I, Chang SY, Bae ON, Kim JK. Enhanced acute anti-inflammatory effects of CORM-2-loaded nanoparticles via sustained carbon monoxide delivery. Eur J Pharm Biopharm. 2016; 108: 187–195. https://doi.org/10.1016/j.ejpb.2016.09.008.
10. Wei Y, Chen P, de Bruyn M, Zhang W, Bremer E, Helfrich W. Carbon monoxide-releasing molecule-2 (CORM-2) attenuates acute hepatic ischemia reperfusion injury in rats. BMC gastroenterol. 2010; 10: 42. https://doi.org/10.1186/1471-230X-10–42.
11. Wilson JL, Fayad Kobeissi S, Oudir S, Haas B, Michel B, Dubois Randé JL, Ollivier A, Martens T, Rivard M, Motterlini R, Foresti R. Design and synthesis of new hybrid molecules that activate the transcription factor Nrf2 and simultaneously release carbon monoxide. Chemistry. 2014; 20: 14698–14704. https://doi.org/10.1002/chem.201403901.
12. Yabluchanskiy A, Sawle P, Homer–Vanniasinkam S, Green CJ, Foresti R, Motterlini R. CORM-3, a carbon monoxide-releasing molecule, alters the inflammatory response and reduces brain damage in a rat model of hemorrhagic stroke. Crit Care Med. 2012; 40: 544–552. https://doi.org/10.1097/CCM.0b013e31822f0d64.

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EVALUATION OF SUSCEPTIBILITY OF REFERENCE STRAINS OF MICROORGANISMS TO THE COMBINED ACTION OF ESSENTIAL OILS AND MEXIDOL

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The susceptibility of the reference strains S. aureus ATCC 25923 and E. coli ATCC 25922 to 14 essential oils and their combinations with mexidol (ethylmethylhydroxypyridine succinate) was studied using the disk diffusion method. It was shown that a combination of all essential oils (with the exception of eucalyptus and ginger) with mexidol increases the susceptibility of S. aureus ATCC 25923 to these agents, with the most pronounced effect being observed with oils of lemon, lavender, fir, and rose. The susceptibility of E. coli ATCC 25922 to essential oils of cinnamon, mint, tea tree, rose, eucalyptus, cloves, and sage significantly increases when combined with mexidol, and this effect is the most pronounced when using combinations of mexidol with rose, eucalyptus, and clove oils. The discovered ability of mexidol to increase the susceptibility of microorganisms to essential oils can be a basis for the development of pharmaceutical compositions with these substances in which an enhanced antimicrobial effect will accompanied by an antioxidant activity and low toxicity.

Key words: microorganisms' susceptibility, *S. aureus*, *E. coli*, mexidol, essential oil.

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ОЦІНКА ЧУТЛИВОСТІ ЕТАЛОННИХ ШТАМІВ МІКРООРГАНІЗМІВ ДО КОМБІНОВАНОЇ ДІЇ ЕФІРНИХ ОЛІЙ І МЕКСИДОЛУ

Досліджено чутливість еталонних штамів мікроорганізмів *S. aureus* ATCC 25923 та *E. coli* ATCC 25922 до 14 ефірних олій та їх комбінацій з мексидолом (етилметилгідроксипіридину сукцинатом) за допомогою диск-дифузійного методу. Показано, що комбінування всіх ефірних олій (за винятком евкаліпту та імбиру) з мексидолом збільшує чутливість *S. aureus* ATCC 25923 до цих засобів, причому найбільш виразний ефект спостерігається стосовно олій лимону, лаванди, піхти та троянди. Чутливість *E. coli* ATCC 25922 ло ЕО кориці, м'яти, чайного дерева, рози, евкаліпту, гвоздики і шавлії істотно зростає при їх комбінуванні з мексидолом, причому найбільш виразним такий ефект є при використанні комбінацій мексидолу з оліями троянди, евкаліпту та гвоздики. Виявлена здатність мексидолу підвищувати чутливість мікроорганізмів до ефірних олій може стати основою для розробки фармацевтичних композицій з цими компонентами, в яких посилена антимікробна дія буде поєднуватись з антиоксидантною активністю і низькою токсичністю.

Ключові слова: чутливість мікроорганізмів, *S. aureus*, *E. coli*, мексидол, ефірна олія.

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Essential oils (EOs) are volatile secondary metabolites that give plants a characteristic aroma and taste. They are produced by more than 17,500 species of many plants families, but only about 300 of them are commercialized [15]. Having a content of two or three main components at the level 20–70 %, EOs are complex mixtures of terpenes, terpenoids and other compounds [15]. A known feature of EO is their antimicrobial action, which is characterized by a wide spectrum, is not attenuated at the presence of protein,