PEPTIDE TOXINS TARGETING ION CHANNELS AS CYTOPROTECTIVE AGENTS IN ISCHEMIA-REPERFUSION INJURY OF EPITHELIAL CELLS

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Ischemia-reperfusion injury (IRI) is a complex process accompanying cessation of blood supply to an organ or tissue followed by subsequent restoration of blood circulation. The IRI is especially prominent in surgery and organ transplantation. One of the strategies for reducing organ and tissue damage during transplantation is regulation of intracellular ion concentrations. Maintenance of ion concentrations in the cell during damage development can be controlled by influencing voltage-dependent ion channels with certain types of compounds. We propose the peptide toxins tropic to calcium (omega-hexatoxin-Hv1a) and sodium (mu-agatoxin-Aa1a) voltage-dependent ion channels as potential agents reducing IRI. The toxins were obtained using solid-phase peptide synthesis. The IRI modeling for evaluation of the action of toxins was carried out on a culture of epithelial cells CHO-K1 during their incubation under conditions of hypoxia and nutrient deprivation followed by subsequent replenishment of the nutrient medium. The level of cell death, concentrations of calcium, sodium, potassium ions, and pH were recorded using a multimodal plate reader and fluorescent dyes. Experiments have shown that regardless of different mechanisms of action, both toxins reduced the development of CHO-K1 cell death by changing ion concentrations and maintaining the pH level.

Keywords: ischemia-reperfusion injury; voltage-dependent calcium channels; voltage-dependent sodium channels; peptide toxin

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INTRODUCTION

Ischemic injury is a complex process accompanying cessation of blood supply to an organ of tissue. Subsequent reoxygenation and nutrient replenishment paradoxically aggravate ischemic injury and lead to ischemia-reperfusion injury (IRI). The problem of reperfusion injury is particularly prominent in surgery and organ transplantation. Reperfusion injury is the main cause of complications and even mortality in surgical operations or transplantation of the liver, kidneys, lungs, ovaries, etc. [1–3].

Ischemia occurs as a result of the removal of the donor organ (warm ischemia), storage in a cold preservative solution (cold ischemia) and during engraftment, causing profound tissue hypoxia, cellular metabolic imbalance, and microvascular dysfunction [3–5]. Reperfusion injury in this case occurs several hours or days after the initial ischemia during organ engraftment and manifests itself in allograft dysfunction [3, 6].

Reperfusion injury is the result of several interrelated processes. A decrease in tissue oxygenation changes cellular metabolism due to a decrease in oxygen supply and removal of end products. The transition to anaerobic cellular metabolism results in decreased ATP production, lactate accumulation, and intracellular acidosis. This causes accumulation of Na^+ and Ca^{2+} ions, activation of Ca^{2+}-dependent proteases (calpains), destabilization of lysosomal membranes with leakage of lysosomal enzymes, destruction of the cytoskeleton, and inhibition of the activity of membrane-bound Na^+/K^+-, Na^+/H^+-, and Ca^{2+}-ATPases. Restoration of blood flow (reperfusion) is also associated with the production of reactive oxygen species (ROS). ROS can alter membranes and DNA through lipid peroxidation and protein carbonylation. Excess Ca^{2+} and ROS in combination with mitochondrial dysfunction cause opening of the mitochondrial permeability transition pore (mPTP) and subsequent release of cytochrome *c*, mitochondrial DNA, and succinate [7, 8]. Moderate ischemia and reperfusion in organ transplantation can lead to cellular dysfunction, which can be mitigated by activation of repair systems to control ROS production. However, prolonged and severe ischemia and reperfusion cause cell death by apoptosis and necrosis [2].

Strategies to reduce IRI in organ transplantation are currently largely related to elucidation of the physiological and molecular pathways activated during IRI. One such strategy is regulation of ion concentrations.

Although the role of voltage-dependent ion channels in the development of IRI in the heart and brain is well known [2, 9], certain evidence exists that that voltage-dependent ion channels are also

expressed in non-excitable cells [10, 11]. However, their putative physiological functions and regulation of their activity in non-excitable cells, especially during the development of IRI, remain the subject of active discussion, because voltage-dependent ion channels are primarily molecular determinants of cellular excitability [12, 13]. In this case, voltage-dependent ion channels in non-excitable cells mediate key cellular functions through intracellular biochemical mechanisms rather than fast electrical signaling [14]. In this context, voltage-dependent calcium and sodium ion channels, which play an important role in modulating the driving force of Ca^{2+} and Na^{+} influx into non-excitable cells attract particular interest. The channels are also involved in the regulation of cell volume by initiating the loss of intracellular K^{+} and concomitant water loss, leading to a decrease in cell volume [15].

We propose to use arthropod toxins (omega-hexatoxin-Hv1a from the spider *Hadronyche versuta* as a calcium channel blocker (Uniprot: TO1A_HADVE) and mu-agatoxin-Aa1a from the spider *Agelenopsis aperta* (Uniprot: T5G1A_AGEAP) as a sodium channel modulator) as regulators of the activity of voltage-dependent calcium and sodium channels in non-excitable cells. These toxins belong to the knottin family; members of this family are characterized by high affinity for the target channels and increased bioavailability due to stability with respect to temperature, proteases, and pH. The mechanism of mu-agatoxin-Aa1a action has been described in more detail previously [16]. This paper presents results of a comparative analysis of the effect of mu-agatoxin-Aa1a and omega-hexatoxin-Hv1a on cell survival in a model of IRI, with an emphasis on key parameters such as cell death and dynamics of ions and pH.

MATERIALS AND METHODS

Peptide Synthesis of Toxins, Their Analysis, Purification, and Folding

The synthesis of toxins was carried out on a ResPep SL peptide synthesizer (Intavis, Germany) in accordance with the standard solid-phase synthesis protocol [17]. Fmoc-protected amino acids (Intavis) were used for the peptide synthesis, and HBTU (Chemical Line, Russia) was used as an activator.

The toxins were analyzed on a Dr. Maisch Luna C18 column using a Shimadzu LC-20AD XR HPLC system (Shimadzu, Japan) with an SPD-20A detector and a gradient elution protocol using 95% solution A (deionized water) and 5% solution B (acetonitrile, Kriokhrom, Russia). For mass spectrometric analysis, a hardware and software complex of the MALDI-TOF MS FLEX series (Bruker Daltonics, Germany) was used. Toxins were purified using the Bio-Gel P-4 sorbent on an Econo-Column 1×30 cm column (Bio-Rad, USA), and a chromatography HPLC system (NGC Quest™ 10, Bio-Rad).

Toxin folding was performed in 0.1 M Tris-HCl buffer containing 10 mM reduced and 1 mM oxidized glutathione (all reagents from PanEco, Russia) at pH 8.0 and 4°C with constant stirring for 24 h [18].

Cell Culture and Experimental Conditions

A cell culture of epithelial origin CHO-K1 (Russian Collection of Vertebrate Cell Cultures, Russia) was used in this study. The cells were cultured in DMEM/F12 medium (PanEco) and passaged every 3–4 days using 0.25% trypsin (PanEco).

Before the experiment, the cells were transferred to 96-well plates and left until they reached the exponential growth stage. For IRI modeling, cells were first placed in conditions of the reduced oxygen and nutrient supply (DMEM medium with 1 g/l glucose (PanEco) and 1% fetal bovine serum (Biosera, France)) in an incubator with 1% O_2 and 5% CO_2 (Binder, Germany) for 3 h ischemia, then in conditions of normal nutrient and oxygen content (standard culture medium with 10% serum at 18.6% O_2 and 5% CO_2) for 3 h reperfusion [19]. Toxins dissolved in deionized water were added at the beginning of the reperfusion stage at a final concentration of 50 nM.

Fluorescence Analysis

After 3 h reperfusion, fluorescent dyes Yo-Pro 1 and PI were added to the medium at a final concentration of 1 μM [20] to detect cell death, and indicators of calcium ion concentrations, Rhod 2 AM 500 nM [21], sodium, ION NaTRIUM Green 2 AM 500 nM [22], potassium, ION Potassium Green 2 AM 500 nM [23] were added to the medium to detect concentrations of corresponding ions. The pH dynamics was analyzed using 1 μM BCECF AM dye [24], which was added immediately after ischemia and 30 min, 1.5 h, and 3 h after the start of reperfusion. All dyes were incubated with the cells for 20 min in the dark. Then the medium with dyes was removed, and the wells of the plate were washed twice with warm phosphate-buffered saline (Sigma-Aldrich, USA). Fluorescence was recorded using a CLARIOstar Plus multimodal plate reader (BMG LABTECH, Germany) in 100 μl of buffer in a matrix scanning mode. The results were processed using the MARS program (BMG LABTECH). The cell concentration was counted using a Goryaev chamber and the data were normalized to 100,000 cells in MS Excel.

Cell Index Analysis

The response of cells to IRI modeling and the effect of toxins were assessed by changes in their "spreading" under changing conditions according to the dynamics of the cell index in real time. For this purpose, an xCELLigence RTCA-S16 cell analyzer (ACEA Biosciences, USA) was used [25]. After passage, the cells were seeded into a 16-well plate

in a standard medium (with fixed zero point). Upon reaching the exponential stage, the medium was replaced to simulate ischemia and transferred to an incubator with 1% O_2. After 3 h, the medium was replaced for reperfusion and transferred to normal conditions. Toxins were also added to the reperfusion medium at a concentration of 50 nM. The cell index was recorded throughout all manipulations. The group with normal conditions was not exposed to IRI.

Statistical Data Processing

All experiments were performed in triplicates with at least three groups in each triplicate. The asymmetry and kurtosis criteria were used to determine the nature of the distribution. The statistical significance of differences was evaluated using the Mann-Whitney test (due to the small sample size); data processing was performed in the Origin program (OriginLab, USA). The Bonferroni test was used to eliminate the effect of multiple comparisons. Differences between groups were considered statistically significant at $p \leq 0.01$.

RESULTS

Both toxins were obtained by solid-phase peptide synthesis with a purity of over 90% (Fig. 1). After the refolding stage the toxins acquired a structure characteristic of knottins in the form of three beta-sheets due to disulfide bridge formation. Then, the effect of the toxins was analyzed by modeling IRI in the CHO-K1 cells culture.

Analysis of the level of cell death (with Yo-Pro 1 staining as an indicator of early apoptosis [26] and PI as an indicator of late apoptosis and/or necrosis [27]) in the CHO-K1 cells exposed to IRI conditions showed a 2.5-fold increase in both indicators as compared with cells maintained under normal conditions throughout the experiment (Fig. 2).

Cell death analysis at the reperfusion stage has shown that the addition of the mu-agatoxin-Aa1a toxin, a modulator of sodium potential-dependent channels, maintained the state of the cells at the level of cells with normal cultivation conditions. The omega-hexatoxin-Hv1a toxin also decreased cell death, but the effect was more pronounced with respect to early apoptosis.

Another indicator that also reflects the response of cells to external influences, manifested in a change in cell adhesion, is the cell index (Fig. 3).

The change in the cell index was registered in four conditions: normal culture conditions (normoxia), normoxia with the addition of toxins, experimental conditions of IRI, and IRI with toxin addition

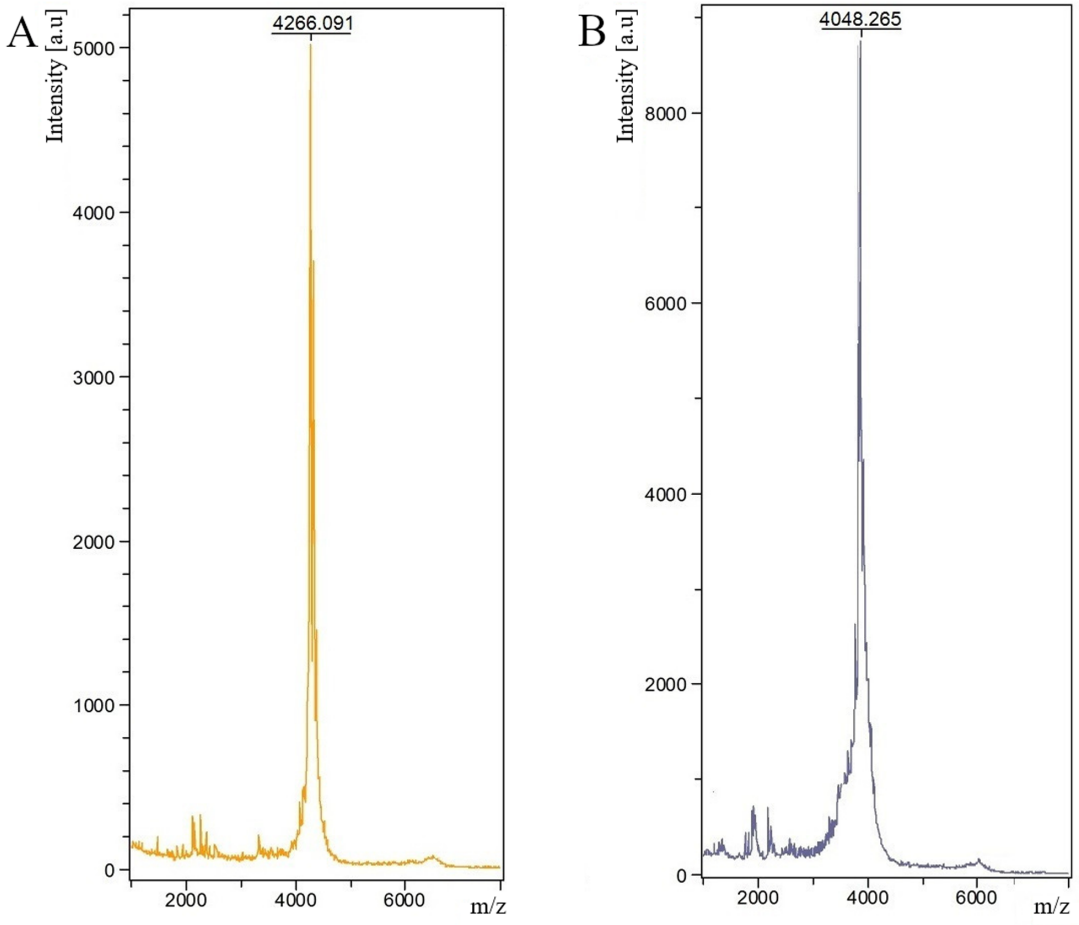

Figure 1. Mass spectrograms of mu-agatoxin-Aa1a (**A**) and omega-hexatoxin-Hv1a (**B**).

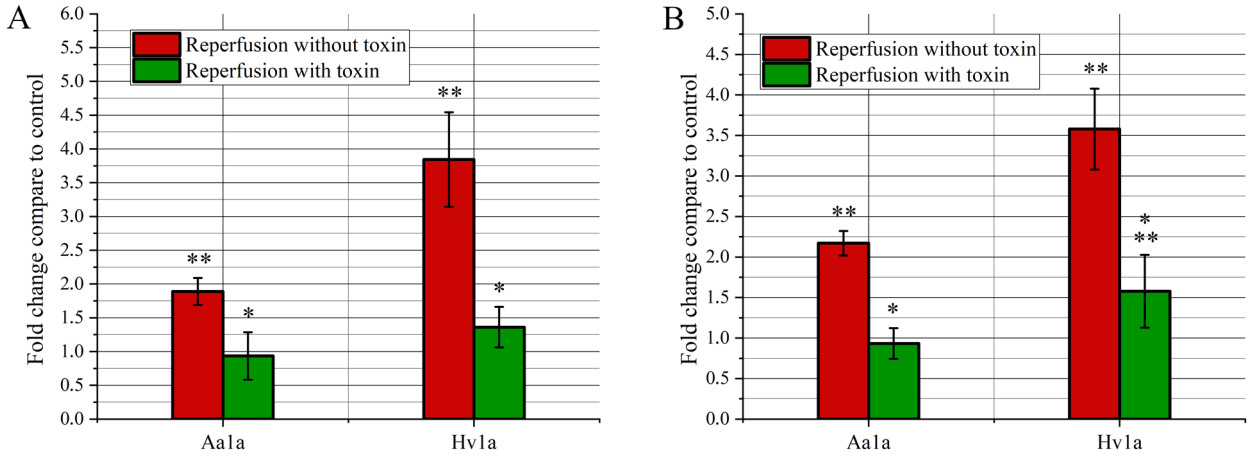

Figure 2. Detection of cell death by staining with Yo-Pro 1 (**A**) and PI (**B**) at the stage of reperfusion in the presence of toxins and without them in the CHO-K1 cell culture. The data are presented as the ratio of fluorescence intensity in relative units of the experimental group (experiment) to the group with normal culture conditions (control) (* – statistically significant difference from the "reperfusion without toxin" group, $p<0.01$, ** – statistically significant difference from normal culture conditions, $p<0.01$).

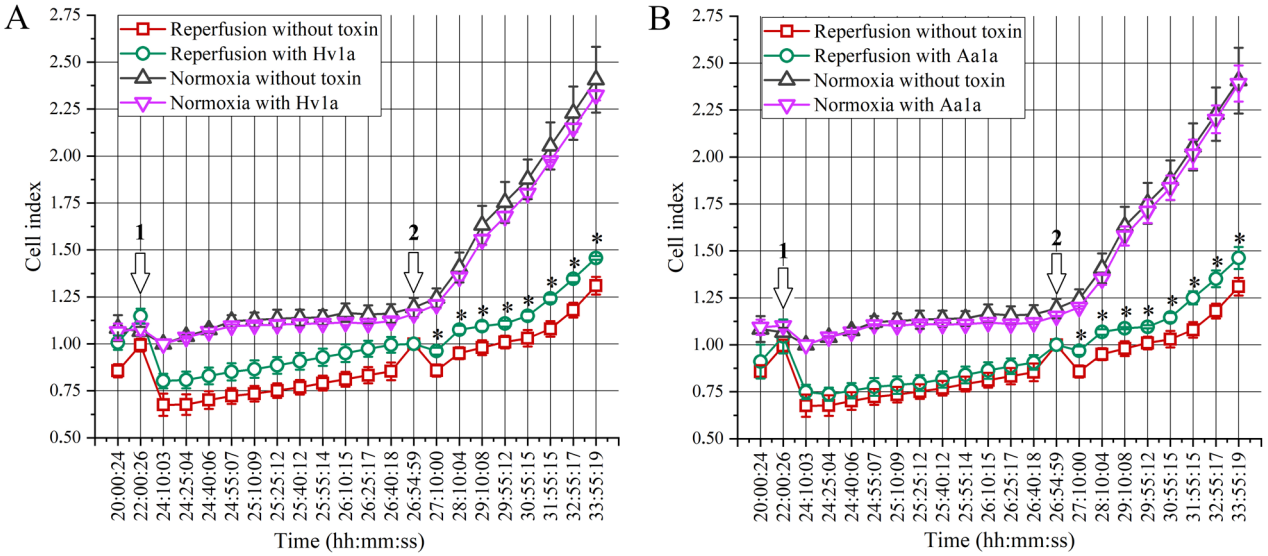

Figure 3. Dynamics of the cell index as an indicator of changes in cell adhesion under the influence of various conditions of CHO-K1 cell cultivation during IRI modeling and under normoxia in the presence of omega-hexatoxin-Hv1a (**A**) and mu-agatoxin-Aa1a (**B**) and without toxins (point 1 – the onset of ischemia (for the "normoxia" group – a change of medium), point 2 – the onset of reperfusion; the reperfusion group was normalized by point 2, the normoxia group was normalized by point 1; * – statistically significant difference from the "reperfusion without toxin" group, $p<0.01$).

at the reperfusion stage. It should be noted that the toxins themselves do not affect the cell spreading and this can be interpreted as the absence of their effect on the overall cell viability (cell growth continues in the same dynamics as under normal conditions). However, when an ions imbalance in the cells occurs (as in the case of IRI modeling) the effect of the toxins becomes evident.

Separate consideration of the cell index change during changes in conditions characteristic of IRI, has shown that at the onset of ischemia (Fig. 3, point 1), the index sharply decreases, but then demonstrates continuous slow increase, thus indicating cell adaptation to reduced serum, glucose, and oxygen parameters.

At the reperfusion stage (Fig. 3, point 2), when the content of nutrients and oxygen is restored, the index also decreases sharply, while the return to the initial value (before the start of reperfusion) occurs within 3 h. In the presence of toxins, the situation is different. Addition of mu-agatoxin-Aa1a to the reperfusion medium leads to a slight decrease in the index within the first 20 min, then the index quickly normalizes to the initial value and continues to grow with the same dynamics. A similar picture is noted in the case of omega-hexatoxin-Hv1a addition: the index also normalizes relatively quickly and continues to grow, while in the group with reperfusion without toxins, the increase in the index slows down.

Analysis of the dynamics of calcium, sodium, and potassium ions during the development of IRI indicates that at the ischemia stage, the concentration of all ions significantly increases (Fig. 4).

At the reperfusion stage, the calcium ion concentration also remained elevated at the same level as during ischemia (Fig. 4A), and the sodium and potassium ion concentration decreased (Fig. 4B,C). In this case, the sodium concentration decreased to a level below normal conditions, and the potassium concentration, despite the decrease relative to the ischemic stage, remained elevated versus normal culturing conditions. The effect of toxins, despite their similarity in reducing cell death, differed. For example, omega-hexatoxin-Hv1a behaved in accordance with its biological function. Being a calcium channel blocker, it reduced the calcium ion concentration by almost 4 times relative to the group without the toxin; this was significantly lower than the concentration under normal conditions. The sodium and potassium concentrations, which were reduced at the reperfusion stage in the presence of the toxin, decreased even more, so that the concentrations of both ions were lower than the concentrations under normal conditions. During addition of the mu-agatoxin-Aa1a toxin to the reperfusion medium, the calcium concentration decreased, but to the level of normal conditions (in contrast to omega-hexatoxin-Hv1a). The concentration of sodium ions also decreased versus ischemia, but in contrast to reperfusion without the toxin, where the concentration significantly decreased, the decrease occurred up to the normal level. At the same time, no changes in the potassium ion concentrations were noted.

The pH level is directly related to the intracellular ion concentrations. Figure 5 shows that acidosis develops at the ischemia stage (0 min reperfusion).

During subsequent reperfusion, a significant increase in pH occurred, following by a decrease throughout the reperfusion stage to a level below normal conditions. The omega-hexatoxin-Hv1a toxin prevented a sharp increase in pH, maintaining this parameter at a reduced level throughout the reperfusion stage (Fig. 5A). The mu-agatoxin-Aa1a toxin

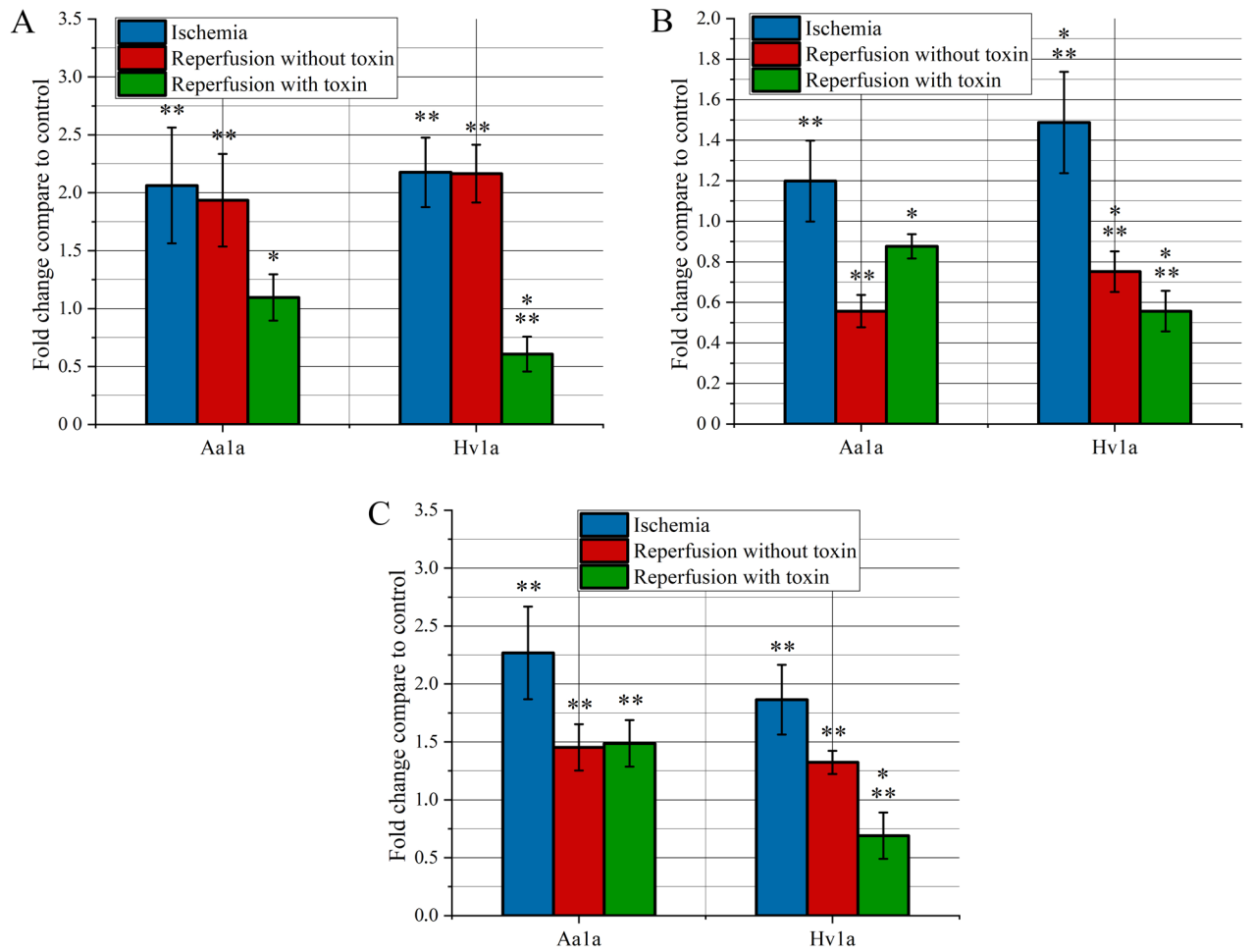

Figure 4. Concentrations of calcium (**A**), sodium (**B**), and potassium (**C**) ions at different stages of IRI modeling in the CHO-K1 cell culture with and without toxins as a ratio of fluorescence intensity in relative units of the experimental group (experiment) to the group with normal cultivation conditions (control) (* – statistically significant difference from the "reperfusion without toxin" group, $p<0.01$, ** – statistically significant difference from normal cultivation conditions, $p<0.01$).

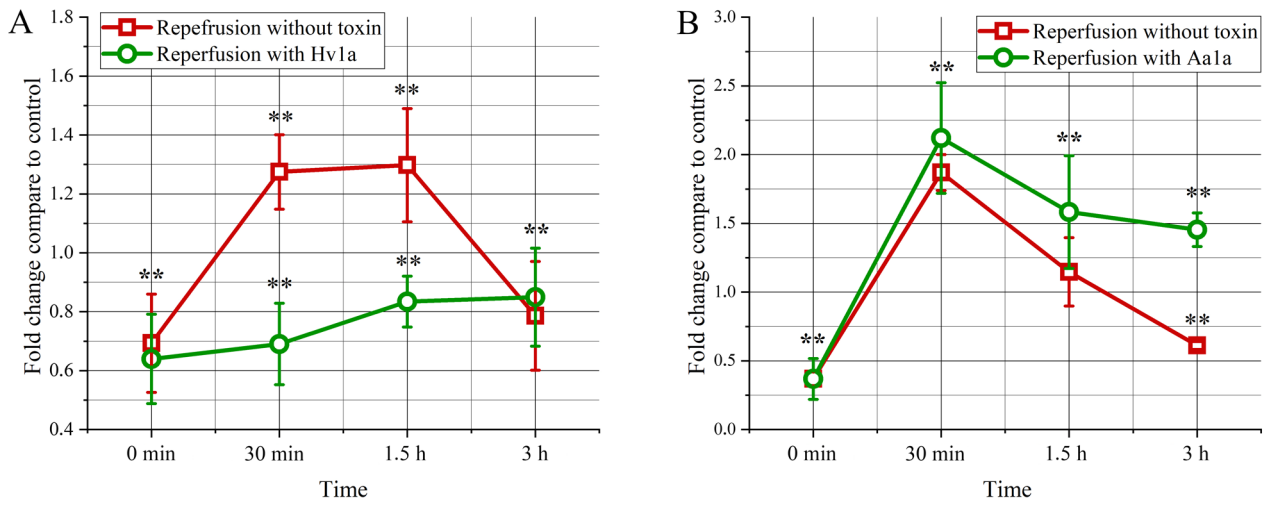

Figure 5. Dynamics of pH during IRI in the CHO-K1 cell culture with and without toxins (**A** – omega-hexatoxin-Hv1a; **B** – mu-agatoxin-Aa1a) (** – statistically significant difference from normal conditions, $p<0.01$).

behaved differently (Fig. 5B). After its addition to the medium at the beginning of the reperfusion stage, the pH level also increased, but was then maintained at an elevated level throughout the reperfusion stage (in contrast to omega-hexatoxin-Hv1a).

DISCUSSION

The role of ions in the development of IRI in excitable cells has long been proven [28]. However, the involvement of voltage-dependent ion channels in the development of this pathology still needs detailed research. The canonical role of voltage-dependent calcium and sodium channels in impulse electrogenesis and conductivity in excitable cells is well known and relatively well studied [29–32]. However, in addition to the expression of calcium and sodium channels in neurons, myocytes, cardiomyocytes, and other excitable cells, there is convincing evidence that several subtypes of voltage-dependent channels (the same channels that support electrogenesis in nerve cells, muscle cells, and cardiac myocytes) are expressed in cell types that are considered non-excitable [12, 33]. Moreover, these channels play an important role in functionally significant processes. The expression of sodium and calcium channels is not static but dynamic in nature depending on the state of the cells. For example, a significant increase in the expression of the sodium channel Nav1.5 was noted in reactive astrocytes at the border of cicatricial injury in an *in vitro* model [34]. Differentiation of human fibroblasts into myofibroblasts in pathological conditions is also accompanied by *de novo* expression of Nav1.5 [35]. In addition, changes in Nav1.5 expression were also noted in Muller cells and phagosomes of activated macrophages [36, 37]. A similar picture was found with the expression of calcium voltage-dependent channels. Initial studies determined the role of Cav channels in excitation-contraction coupling in muscles and synaptic transmission in neurons. In addition to the relatively well-characterized functions of Cav channels in excitable cells, numerous studies have demonstrated the functional expression of these channels in normal non-excitable cells such as embryonic and stem cells [38, 39], lymphocytes [40], retinal pigment epithelium [41], and a number of epithelial cancer cell types [42]. Furthermore, the use of calcium channel blockers in the treatment of hypertrophic wound healing disorders highlights the role of the voltage-gated channels in the skin [43], as does the increased expression of Cav1.2 in valves from patients with calcific aortic stenosis [44].

All these data underline the importance of voltage-gated calcium and sodium channels in non-excitable cells, especially in the development of pathological conditions. IRI is one such condition and, most importantly, in organ transplantation.

It is known that reperfusion after short-term ischemia results in both apoptosis and necrosis [45, 46]. Cells switch aerobic metabolism to anaerobic due to decreased oxygen supply and this results in decreased ATP production and intracellular acidosis due to lactate formation. This in turn leads to inhibition of Na^+/K^+-ATPase accompanied by intracellular accumulation of Na^+ ions and water, followed by cell swelling. Decreased Ca^{2+} excretion causes intracellular Ca^{2+} accumulation occurs, activation of Ca^{2+}-dependent proteases, and Ca^{2+} entry into mitochondria. In mitochondria, Ca^{2+} overload is responsible for the generation of reactive oxygen species. This leads to the opening of the mitochondrial mPTP pore after reperfusion (under conditions of increased concentrations of Ca^{2+} ions) [9]. The massive Na^+ influx into the cell, which continues during reperfusion due to the displacement of excess H^+ ions through the Na^+/H^+ exchanger, drives abnormal operation of Na^+/Ca^{2+} exchanger,

which functions in the reverse mode [47, 48], thus aggravating Ca^{2+} accumulation. Subsequent processes triggered by Ca^{2+} and Na^{+} ions lead to cell death. This problem can be solved by using drugs targeting ion channels, through which ions are transported outside/inside the cell.

Studies of the role of voltage-dependent channels in organ transplantation and the prospects for using this strategy to reduce ischemia-reperfusion injury have been conducted since the early 1990s. For example, it was shown that calcium channel blockers verapamil, bepridil, and nifedipine reduced apoptosis in the renal tubules in a rat model of IRI [49]. The use of verapamil in intestinal epithelial cell ischemia also reduces the level of death in an *in vitro* model [50]. The antioxidant mechanism of neuroprotective activity of verapamil was demonstrated in a rat brain IRI model [51]. Limited information was obtained regarding potential-dependent sodium channels. For example, the use of lidocaine as a sodium channel blocker in a rat kidney IRI model decreased cell death [52].

We used arachnid venom peptide toxins to reduce IRI. In contrast to synthetic drugs Arthropod toxins have evolutionarily high affinity for specific types of ion channels, [53]. We focused on two toxins: omega-hexatoxin-Hv1a from the spider *Hadronyche versuta* and mu-agatoxin-Aa1a from the spider *Agelenopsis aperta*. These toxins have different mechanisms of action: omega-hexatoxin-Hv1a is an inhibitor of calcium channels of the Cav1.2, Cav2.1, and Cav2.2 types, it blocks the conductivity of calcium ions [54], while mu-agatoxin-Aa1a is a modulator of sodium channels. Modification of sodium channels by the toxin leads to an increase in the sensitivity of these channels to the membrane potential due to a shift in the activation curve to a more negative potential. This leads to an increased probability of sodium channel opening when the membrane potential changes. It is also worth considering that the study of the effect of toxins has been carried out using the CHO-K1 cells, which belongs to non-excitable cells and express both sodium and calcium potential-dependent channels.

Addition of both toxins to the nutrient medium at the reperfusion stage decreased the rate of cell death (Fig. 2) and omega-hexatoxin-Hv1a had a more pronounced effect. Cell spreading as one of the parameters of cell response to exposure to toxins under reperfusion conditions also increased relative to the control group without toxins (Fig. 3). In the context of this parameters both toxins acted similarly, and also they did not affect the cell culture when added to the nutrient medium without inducing cell death.

Since the key processes in the IRI development are associated with changes in the concentration of Ca^{2+}, Na^{+}, and K^{+} ions, changes in these parameters were recorded separately (Fig. 4). It is evident that the change in ion concentrations, both at the ischemia stage and at the reperfusion stage, occurs in accordance with canonical mechanisms: the concentration of all ions increases at the ischemia stage, while at the reperfusion stage the concentration of Ca^{2+} remains elevated, and the concentrations of Na^{+} and K^{+} decrease to a level close to physiological conditions (K^{+} ions) or even below the physiological level (Na^{+} ions). In this case, toxins behave differently, and this difference is due to mechanisms of their action. The calcium channel blocker omega-hexatoxin-Hv1a significantly reduces the concentration of Ca^{2+}, Na^{+}, and K^{+} ions to a level significantly below normal one. At the same time, the sodium channel modulator mu-agatoxin-Aa1a reduces the concentration of Ca^{2+} and Na^{+} ions to the physiological level, and does not influence the K^{+} concentration (the level corresponds to the control without the toxin).

In the context of IRI, the development of the pH paradox is especially important. It is associated with onset of ischemia-induced acidosis developed by H^{+} accumulation due to switch into glycolytic energy production in the cell. During subsequent reperfusion, when the restored blood flow delivers nutrients, alkalosis develops, when the pH level sharply increases to a level above the physiological one and the cells begin to die [55]. We see this in our experiments (Fig. 5). Immediately after ischemia, a decrease in pH is noted. During the first 30 min of ischemia, the pH level increases and remains above normal, slowly decreasing to the initial level in the next 2.5 h. This is accompanied by increased cell death. Addition of toxins influences the picture. Omega-hexatoxin-Hv1a prevents an increase in pH throughout the reperfusion stage (Fig. 5A), while mu-agatoxin-Aa1a, despite the initial increase in pH as in the control group, maintains it an elevated level throughout the reperfusion (Fig. 5B).

Thus, IRI modeling in the culture of epithelial cells CHO-K1, is accompanied by cell death processes are triggered by an increase in the concentration of Ca^{2+} ions and the pH level during reperfusion after acidosis developed during ischemia. The toxin omega-hexatoxin-Hv1a acts as a blocker of calcium channels, one of the route for Ca^{2+} ions entry to the cell during reperfusion. The channel blockade leads to a decrease in the Ca^{2+} concentration, which in turn affects Na^{+} ions. Blocking calcium channels, the toxin causes a decrease in Ca^{2+}; as a result Na^{+} and H^{+} concentrations do not increase, and the pH level remains lowered after ischemia. All this prevents the development of events leading to apoptosis and necrosis.

Another scenario is realized in the case of the sodium channel modulator mu-agatoxin-Aa1a. Results of various studies show that the addition of sodium channel blockers increases cell survival during the IRI development [52, 56]. However,

in our case, the toxin acts as a modulator that maintains the open state of the channel by reducing its sensitivity to the membrane potential. The activity of sodium channels in non-excitable cells leads to Na^+ influx, which leads to the reverse operation of the Na^+/Ca^{2+} exchanger, Ca^{2+} import, and modulation of calcium signaling. At the same time, at the ischemia stage, the membrane potential decreases due to the work of sodium channels, and at the reperfusion stage, repolarization begins, leading to sodium channel closure [57]. Maintaining the sodium channel open state should lead to an increase in cell death. However, under our experimental conditions, a significant decrease in cell death was noted. In this case, an analogy can be drawn between our experiment and the works of other researchers. Thing is that with the development of injuries or other pathological conditions, the expression of sodium channels increases [34, 35]. In our case, compensation for the of Na^+ influx into the cell occurs not by increasing channel expression, but by maintaining these channels in an open state. As a result, the outflow of Na^+ occurs not only due to exchange for H^+ through the corresponding exchanger, but also due to the outflow of Na^+ through open voltage-dependent channels, while the outflow occurs at a significantly lower rate. Another important point is the rate of toxin binding to the channel. As we see in Figure 5, the toxin begins to work only 30 min after the onset of reperfusion, which is manifested in a slow decrease in pH over the next 2.5 h.

CONCLUSIONS

Thus, despite the different mechanism of action, both toxins act as cytoprotective agents during ischemia-reperfusion damage of epithelial tissues. The obtained data can be used to reduce allograft dysfunction in organ transplantation.

FUNDING

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COMPLIANCE WITH ETHICAL STANDARDS

No human/animal was directly involved in the sampling process during this study. Local Russian regulations do not require approval for the use of cell line biomaterials for scientific research (Federal Law of June 23, 2016 no. 180-FZ).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ПЕПТИДНЫЕ ТОКСИНЫ, НАЦЕЛЕННЫЕ НА ИОННЫЕ КАНАЛЫ, КАК ЦИТОПРОТЕКТОРНЫЕ АГЕНТЫ ПРИ ИШЕМИЧЕСКИ-РЕПЕРФУЗИОННОМ ПОВРЕЖДЕНИИ ЭПИТЕЛИАЛЬНЫХ КЛЕТОК

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Ишемически-реперфузионное повреждение (ИРП) — совокупность процессов и событий, сопровождаемая нарушением кровоснабжения в ткани или органе с последующим восстановлением кровотока. Особо остро проблема ИРП стоит в хирургии и трансплантологии. Одна из стратегий снижения повреждения органов и тканей при трансплантации — регуляция внутриклеточных концентраций ионов. Поддержание концентрации ионов в клетке во время развития повреждения можно контролировать, воздействуя на потенциал-зависимые ионные каналы определёнными типами соединений. Мы предлагаем снижать ишемически-реперфузионное повреждение при помощи пептидных токсинов, тропных к кальциевым (омега-гексатоксин-Hv1a) и натриевым (мю-агатоксин-Aa1a) потенциал-зависимым ионным каналам. Токсины были получены с использованием твердофазного пептидного синтеза. Моделирование ИРП при действии токсинов проводили на культуре клеток эпителиального происхождения CHO-K1 при инкубации в условиях гипоксии и депривации питательных веществ с последующим восстановлением питательной среды. Уровень клеточной гибели, концентрации ионов кальция, натрия, калия и уровень pH фиксировали с использованием мультимодального планшетного ридера и флуоресцентных красителей. В итоге, оба токсина, несмотря на разный механизм действия, снижают развитие клеточной гибели CHO-K1 за счёт изменения концентраций ионов и поддержания уровня pH.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: ишемически-реперфузионное повреждение; потенциал-зависимые кальциевые каналы; потенциал-зависимые натриевые каналы; пептидный токсин

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