Organic Chemistry
Current Research

Kasumov et al., Organic Chem Curr Res 2015, 4:1
DOI: 10.4172/2161-0401.1000136

Review Article Open Access

On the Mechano-Chemiosmotic Mechanism of Action of Guanidinies on Functional Activity of Mitochondria and Aging

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Abstract

Functional activity of mitochondria in the cell is fully regulated by a complex mixture of dissolved in water substances of the cytosol compound. The concentrations of these substances are different, which is important in processes such as osmoregulation and signaling both of a cell and of mitochondria for ATP synthesis. We studied the action of guanidine on the functional activity of mitochondria. Mitochondria showed a trend to inhibition of oxidative phosphorylation in the presence of different guanidine concentrations. Also, an oscillatory volume changes shrinkage-swelling of mitochondria ¬- are largely abolished and pH changes of incubation medium in addition of ADP are inhibited in the presence of guanidine ion. The mechanism of inhibition of these processes is explained by mechano-chemiosmotic model. In the article, it is discussed that a disruption of the movement of calcium ions (which are moved cyclically between the external environment (cytosol), the matrix and intermembrane space) by various agents, including guanidine leads to disruption of ATP synthesis. We conclude that the L-arginine (2-Amino-5-guanidinopentanoic acid) deficiency in a body leads to a decrease in energy production and, also, it is the cause of aging, type 2 diabetes, cancer and other diseases.

Keywords: Mitochondria; GuHCl, L-arginine; Shrinkage-swelling; Respiration; Mechano-chemiosmotic model; Aging; Cancer

Abbreviations: GuHCl-Guanidine hydrochloride; ETC-Electron transport chain; 2,4-DNP-2,4; Dinitrophenol; NO-Nitric oxide; BSA-Bovine serum albumin; ROS-Reactive oxygen species.

Introduction

One of the main features of living organisms is their ability to extract energy from the environment as a result of redox reactions. ATP is one of the main types of chemical energy for life in biological systems, the synthesis of most of which occurs in the mitochondria. The concentrations of substances in cytosol are different, which is important in processes such as osmoregulation and signaling both of a cell and mitochondria for ATP synthesis [1].

Previously, we suggested that at the oxidative phosphorylation an asymmetric contact between dimers of opposite bc_1 complexes in intracristal space is formed during shrinkage of organelles. Convergence of membranes can provide an "intermembrane" electron transport, which is an important mechanical regulator of electron transfer from Iron-Sulfur cluster-(2Fe-2S) to heme c_1 in respiration. In fact, according to electron microscopy studies in the presence of succinate, mitochondrial membranes are really close to each other [2]. According to other sources of electron microscopy in hypotonic incubation medium at 16°C [3] there is compression of significant portion of mitochondrial cristae. In a study of the fluorescence of mitochondrial proteins, the authors conclude that the compression, happening at lower tonicity at <19°C, is the result of inter-membrane docking [3].

In a previous work [4] we showed that in transferring of mitochondria from resuspension medium at a concentration of 0.4 M sucrose to the medium with a low concentration of sucrose - 0.05 M passive shrinkage of mitochondria was observed, which is characterized in literature as "inter-membrane docking" in the hypo-osmotic conditions [3]. In these conditions mitochondria exhibit a high rate of substrate phosphorylation and respiration as compared to high concentrations of sucrose. At high concentrations of 0.8 M sucrose respiration rate falls sharply, the shrinkage ability of mitochondria is suppressed and phosphorylation activity of mitochondria is reduced, which appears also in changes of the incubation medium pH. The amplitude of the cycle alkalinization - acidification decreases. These data are consistent with mechano-chemiosmotic coupling model [4-6] of electron transport to ATP synthesis, where the following processes are coupled: electron transfer by ETC, proton transfer, the movement of cations, a cyclic low-amplitude swelling-shrinkage and ATP synthesis.

As additional evidence of mechano-chemiosmotic coupling a model system can be created, where mitochondrial shrinkage disappears completely, in adding ADP to energized mitochondria. It is believed that guanidine ions are in protonated form at pH 8 and below, and accumulate in the matrix, advantageously inhibiting respiration in State 3, i.e. after adding ADP [7,8]. If guanidine hydrochloride is to be used as osmotic, then in the hypo-osmotic conditions we should observe no shrinkage when ADP is added to the energized mitochondria. In this regard, we investigated the effect of different concentrations of guanidine hydrochloride (GuHCl) on volume changes, pH changes in incubation medium of mitochondria, and mitochondrial respiration.

It is known [9] that as the alkyl group lengthen; activities are potentiated, amounting to a several hundred-fold increase between guanidine and dodecylguanidine. The introduction into the molecule of a single, uncharged alkyl group favors enhancement of guanidine activity. Wilson and Bonner [7] clearly showed that the guanidine inhibitors act on mung bean mitochondria in a manner differing

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Received: October 24, 2014; **Accepted:** December 06, 2014; **Published**: January 01, 2015

Citation: Kasumov EA, Kasumov RE, Kasumova IV (2015) On the Mechano-Chemiosmotic Mechanism of Action of Guanidinies on Functional Activity of Mitochondria and Aging. Organic Chem Curr Res 4:136. doi:10.4172/2161-0401.1000136

slightly from that observed with rat liver mitochondria. Mung bean mitochondria are less sensitive to the guanidines than rat liver mitochondria.

The results of the experiments with C^{14}-labeled guanidine indicate that the induced P_i binding is paralleled by the binding of guanidine itself. In several experiments, over a wide range of guanidine concentrations, between 1 and 2 moles of guanidine are bound for each mole of P_i binding induced [9,10].

Biguanides inhibit a large scale of metabolic processes which in most cases are membrane-linked. Oxidative phosphorylation is strongly inhibited in that part of the respiratory chain which is located on the substrate site of cytochrome *c*. It is assumed that inhibition is due to physical changes within the membrane phospholipids, rather than a result of direct interaction with functional intermediates of energy conservation [11]. From other data, it was concluded that octylguanidine exerts a direct inhibitory action on soluble F_1 of the ATPase complex [8,12].

Metformin (N,N-Dimethylimidodicarbonimidic diamide) which is the biguanide class in concentrations 10 mM and higher affect the oxidative phosphorylation system by decreasing the rat liver mitochondrial transmembrane potential and increasing the repolarization lag phase. Moreover, metformin exacerbates Ca^{2+}-induced permeability transition pore opening by decreasing the capacity of mitochondria to accumulate Ca^{2+} [13].

On the one hand, the rate of energy-linked mitochondrial swelling and the rate of proton uptake are inhibited during phosphorylation of ADP in the presence of biguanides, and on the other hand, oscillatory volume changes of mitochondria are largely abolished by biguanides [14].

Despite numerous studies the mechanism of action of guanidine on the processes occurring in the mitochondria is still unclear. Understanding the mechanism of action of guanidine derivatives on oxidative phosphorylation is useful in that guanidine derivatives (eg, metformin) have a therapeutic effect on diabetic hyperglycemia [12,15].

Methods

Mitochondria of etiolated hypocotyls of mung bean (*Phaseolus aureus* Roxb., cv. Tadzikskii 1) were taken as an object of investigation. Seedlings were grown on moist filter paper in an environment-control chamber at 25 °C in the dark for a period of 4 days. The isolation medium (0,4 M sucrose, 0,067 M potassium phosphate buffer, 4 mM EDTA, pH 7,4) and batch of material were taken in a ratio of 4:1. Hypocotyls without preliminary homogenization were squeezed through cloth into a breaker containing the isolation medium. Mitochondria were isolated by differential centrifugation [16-18]. The first centrifugation was conducted for a period of 8 min at 15,000g. The residue of mitochondria was washed with isolation medium to which 0,1% BSA was added, centrifuged again (at 15,000g for a period of 6 min), and resuspended in a medium (pH 7,2) containing 0,4 M sucrose and 0,1% BSA. The control incubation medium contained the 0,4 M sucrose, 18 mM KH_2PO_4, and 0,1% BSA (pH 7,2 was established by adding NaOH). In the experimental samples to the incubation medium with 18 mM KH_2PO_4, 0,1% BSA, pH 7,2 different concentrations of GuHCl were added. Sodium succinate was used as oxidation substrate. Sodium glutamate was used to remove oxaloacetic acid formed during oxidation of succinate.

Changes of mitochondrial volume were registered as changes of optical density of a suspension containing ~1 mg of protein at wavelength of 520 nm [19] on a Specord M40 spectrophotometer from Carl Zeiss (Germany) in a vessel with a volume of 3 ml. The decrease and increase of the optical density may be considered as swelling and shrinkage, respectively. The rate of oxygen uptake by mitochondria was determined in a polarographic cell with volume of 1 ml using an enclosed platinium electrode Clark type connected to an OH-polarograph from Radelkis (Hungary).

Hydrogen ion concentrations in mitochondrial suspensions were determined with the aid of an antimony electrode on pH-340 amplifer connected up with self-recording EZ-10 potentiometer from LPP (Czechoslovakia). Experiments were conducted in not less than fivefold biological replication and in more than two analytical replications. The figures cite curves of a typical experiment. Protein was determined according to Lowry et al. [20].

Adenosine diphosphate (ADP), BSA, succinate, glutamate, and EDTA from Serva (Germany), Guanidinium chloride (GuHCl) from Fluka (Switzerland) were used in the work. Salts were preliminary recrystallized.

Results

Effect of GuHCL on changes in pH of mitochondrial incubation medium: Figure 1 presents the kinetics of changes in pH of the medium in the presence of GuHCl and without one. It can be seen on the figure that introduction of mitochondria to an incubation medium without GuHCl caused its acidification, which disappeared after 1-2 min. Addition of oxidation substrate – succinate in the absence of GuHCl (curve 6) led – as was demonstrated previously [16] - to sharp alkalization of the medium, followed by gradual alkalization. In this way, several phases were discernible in the process of alkalization. Addition of ADP to succinate-energized mitochondria caused sharp acidification of the medium, indicating extrusion of protons. This was rapidly replaced by their transport into mitochondria (or exit of OH- from mitochondria), which resulted in greater alkalization of the medium than at the start. After attainment of the maximum, alkalization was replaced by acidification.

When adding mitochondria in the incubation medium in the presence of guanidine acidification was observed, the amplitude of which rose with the increase of concentration of guanidine. Acidification was slowly replaced by alkalinization, in contrast to the control, where alkalinization occurred faster.

Addition of the oxidation substrate - succinate in the presence of GuHCl 0,025M together with sucrose 0,35M (curve 5) and without sucrose (curve 4), has caused a much weaker alkalization of the medium than in the control sample. At higher concentrations of the GuHCl 0,1M, 0,2M and 0,3M (curves 3, 2 and 1, respectively), acidification of medium was observed at the time of addition of succinate, such acidification being replaced by slow alkalization, the amplitude of acidification increasing in proportion to increase in concentration of the GuHCl. ADP caused slight acidification followed by alkalization only in the presence of GuHCl 0,025M with sucrose 0,35M (curve 5) and without sucrose (curve 4). At greater concentrations of GuHCl 0,1M, 0,2M and 0,3M (curves 3, 2 and 1, respectively) almost no changes under the action of ADP were observed.

Effect of GuHCL on volumetric changes and respiration of mitochondria: Figure 2 presents the kinetic curves of changes in mitochondrial volume in the presence of GuHCl and without. As

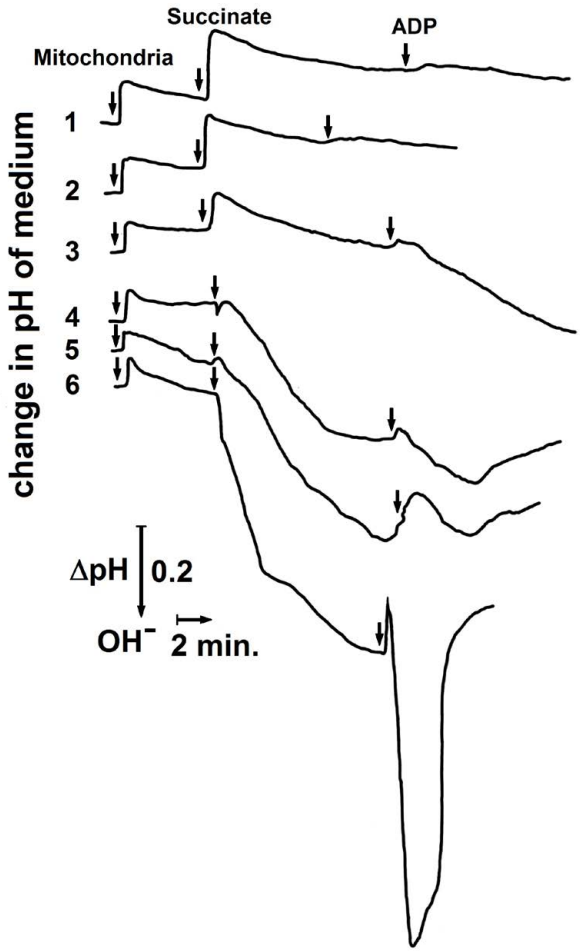


Figure 1: Changes in pH of the incubation medium of mitochondria in the control and in the presence of GuHCl at different concentrations. 1) Control – sucrose 0.4 M; 2) sucrose 0.35 M and 0.025 M GuHCl; 3) 0.025 M GuHCl; 4) 0.1 M GuHCl; 5) 0.2 M GuHCl; 6) 0.3 M GuHCl. The incubation medium contained 18 mM KH_2PO_4, 0,1% BSA and 1mM glutamate, pH 7,2. Arrows indicate addition of 1mg of mitochondrial protein, 2.75 mg of sodium succinate, and 0.24 mg of sodium ADP. Final volume was 1 ml.

can be seen on this figure (curve 1), slight swelling was observed after addition of mitochondria to an incubation medium not containing GuHCl.

Addition of sodium succinate led to momentary mitochondrial shrinkage, replaced by swelling. Thus, mitochondria energized in the presence of succinate are in a swollen state. Addition of ADP to swollen mitochondria caused sharp shrinkage of the organells. This is associated with the phosphorylation: when phosphorylation of ADP to ATP occurs, mitochondria are in a contracted state, while on completion of phosphorylation, they begin to swell to a certain level. It should be noted that this curve is an integral picture and we believe that phosphorylation occurs in cyclic swelling-shrinkage of intracristal space of mitochondria that are not visible in these curves due to low temporary permission from the spectrophotometer.

As can be seen on Figure 2 (curve 2), slight shrinkage was observed after addition of mitochondria to an incubation medium containing GuHCl 0,025M together with sucrose 0,35M. Energization of mitochondria by succinate in these conditions caused a little swelling. When adding ADP to such energized mitochondria there was shrinkage and then swelling, but with very small amplitude compared with the control.

Addition of mitochondria to medium with different concentrations of guanidine caused large changes. As can be seen from Figure 2 (curves 3, 4, 5 and 6) the initial optical density during the addition of mitochondria to mediums with guanidine concentrations (0.025M, 0.1M, 0.2M and 0.3M, respectively) at first was higher than with control and it increased with increasing concentration of guanidine. These data indicate the shrinkage of mitochondria under the influence of different concentrations of guanidine. Subsequently, mitochondria passively swell, and the amplitude of the swelling also depends on the concentration of guanidine, i.e. the greater shrinkage occurs, the greater the passive swelling is.

Energization of mitochondria by succinate in the medium with guanidine 0.025M and 0.1M slows swelling, but with guanidine 0.2M and 0.3M no effect can be observed. Addition of phosphate acceptor - ADP to mitochondria in a medium with guanidine 0.025M, 0.1M, 0.2M and 0.3M causes no shrinkage in comparison with control.

Guanidine inhibited respiration in both States 3 and 4, the increase in the concentration of guanidine increased inhibition of mitochondrial respiration. At the same time inhibition of respiration in the State 3 was larger, indicating the absence of phosphorylation in these conditions.

Figure 3 shows the effect of different concentrations of sucrose and GuHCl on the rate of succinate oxidation by mung bean mitochondria in different metabolic states. It is clearly seen that with increasing concentrations of both sucrose and guanidine ranging from 0.025 M to 0.3 M the rates of the substrate respiration (State 4), and phosphorylating respiration (State 3) decrease. But unlike sucrose in the whole concentration range of guanidine hydrochloride respiration rate in the State 4 was greater than in the State 3, indicating that guanidine inhibits a respiration of mitochondria in State 3 in the presence of ADP.

Discussion

Guanidine at low concentrations did not increase the rate of respiration and mitochondria phosphorylation ability unlike sucrose [1,4,21], but at high concentrations inhibited the functional activity of mitochondria. Our data are in agreement with literature data that guanidines cause an inhibition of mitochondrial respiration [7,9,11] and guanidinies do not inhibit mitochondrial swelling mediated by energy derived from either substrate oxidation or by externally added ATP [22].

It should be noted that volume changes of mitochondria in the medium with sucrose [4] and guanidine (Figure 2) differ sharply. When introducing mitochondria into the medium with 0.025M sucrose the shrinkage was less than with 0.025 M GuHCl, but passive swelling in 0.025 M GuHCl was less than in 0.025 M sucrose. During energization by succinate, also mitochondria swell in 0.025 M GuHCl less than in 0.025M sucrose. Apparently, this is a consequence of a breach of OH^- and phosphate exchange in the presence of guanidine in energization. OH^- does not go out into external medium, but guanidine and phosphate come together into matrix in exchange for protons [9,10], as a result, the acidification of the medium occurs and the degree of acidification depends upon the concentration of guanidine. In response to the addition of ADP to the mitochondria in the presence of succinate respiration was suppressed in State 3, and mitochondrial volume changes as well as pH changes of the medium were not observed.

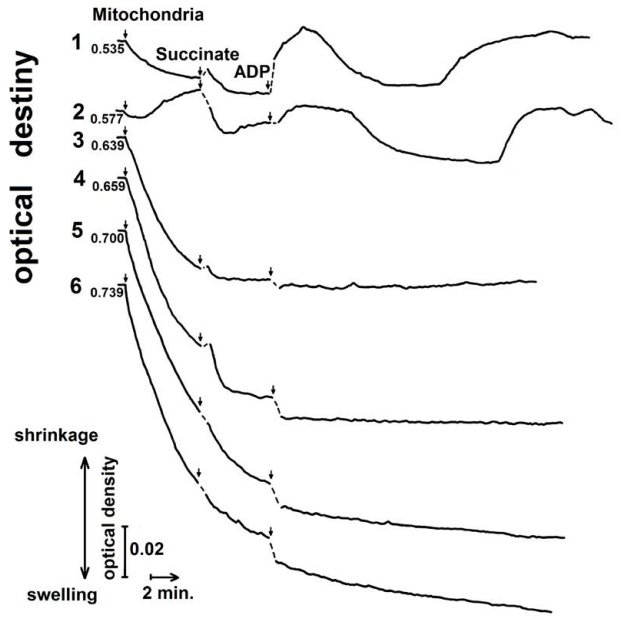


Figure 2: Kinetic curves of changes in mitochondrial volume in the control and in the presence of GuHCl at different concentrations. 1) Control – sucrose 0.4 M; 2) sucrose 0.35 M and 0.025 M GuHCl; 3) 0.025 M GuHCl; 4) 0.1 M GuHCl; 5) 0.2 M GuHCl; 6) 0.3 M GuHCl. The incubation medium contained 18 mM KH_2PO_4, 0,1% BSA and 1mM glutamate, pH 7,2. Arrows indicate addition of 1mg of mitochondrial protein, 2.75 mg of sodium succinate, and 0.24 mg of sodium ADP. Final volume was 3 ml.

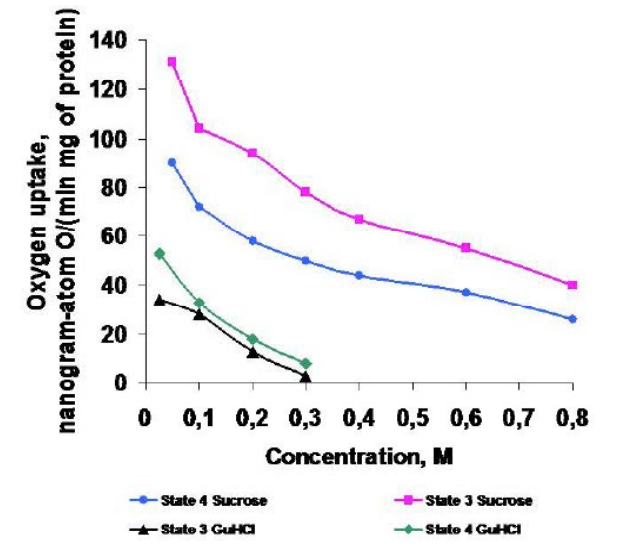


Figure 3: Comparative effects of different concentrations of sucrose and GuHCl on respiration. The dependence of the oxygen uptake in State3 and State4 by mitochondria during succinate oxidation on the concentration of sucrose (curves are taken from the article Kasumov et al. 2012) and GuHCl.

The resulting parallel data - volume changes, changes in pH and respiration in addition to our previous works [16-18], once again show the coupling of these parameters.

The mechanism of inhibition of substrate respiration (State 4), and phosphorylating respiration (State 3) can be explained by mechano-chemiosmotic model [5,6]. This model uses the chemiosmotic model [23] as its basis, and supplements it by dynamic properties characteristic of biological structures with particular attention to a regulatory role of a cyclic low-amplitude swelling-shrinkage of organelles. We have arbitrarily divided description of mechano-chemiosmotic model into two stages in normal conditions and in the presence of guanidine: substrate respiration (State 4), and phosphorylating respiration (State 3)

Stage of energization

We suggest that ATP synthase is a Ca^{2+}/H^{+}- K^{+} Cl^{-} -pump-pore-enzyme complex, in which γ-subunit rotates 360° in steps of 30°, and 90° due to the binding of phosphate ions to positively charged amino acid residues in the N-terminal γ-subunit in the electric field. The coiled-coil b_2-subunits act as ropes that are shortened by binding of phosphate ions to positively charged lysines or arginines; this process is suggested to pull the $\alpha_3\beta_3$-hexamer to the membrane during the energization process.

During energization of mitochondria in the presence of guanidine, OH^{-} from the matrix is exchanged for phosphate ion together with guanidine from the environment [9]. The guanidine exacerbates Ca^{2+}-induced permeability transition pore opening by decreasing the capacity of mitochondria to accumulate Ca^{2+} [13]. Ion pump of ATP synthase pumps the guanidine instead calcium ions into the intracristal space through the ion channel (*c*-ring) of ATP synthase and K^{+} and Cl^{-} ions with the secondary hydration shell into matrix. In the intracristal space a protonation of guanidine and its binding to phospholipids of an inner membrane occurs [8], as a result, the membrane potential is reduced and the repolarization lag phase is increased [13]. Pumping of guanidine into the intracristal space across matrix is an energy-dependent process and it proceeds under polarization of the membrane.

In the presence of guanidine in the matrix the protonated guanidine ions bind instead of calcium ions to γ- and b_2-subunits (F_1) of ATP synthase [8,12] then the rotation of γ-subunit and the twisting of b_2-subunits under action of phosphate ions are absent.

This stage corresponds to the state of cyclic conformational change of mitochondria: phosphate-induced conversion of energized membrane into energized twisted state of inner membrane, as described by Harris et al. [2]. But in the presence of guanidine a phosphate-induced conversion of an energized membrane into energized twisted state of the inner membrane does not occur.

Stage of de-energization

ATP is synthesized during the reverse rotation of the γ-subunit by destabilizing the phosphated N-terminal γ-subunit and b_2-subunits under the influence of Ca^{2+} ions, which are pumped over from storage - intermembrane space into the matrix, during swelling of intermembrane space.

In the presence of guanidine addition of ADP must create a further depolarization of membrane, but ATP synthesis cannot occur due to the absence of phosphate-induced conversion of the energized membrane into energized twisted state. In these conditions a low-amplitude cyclic shrinkage-swelling, in other words oscillatory volume changes of mitochondria are largely abolished [14], repolarization is absent and State 3 respiration is inhibited more than in the State 4.

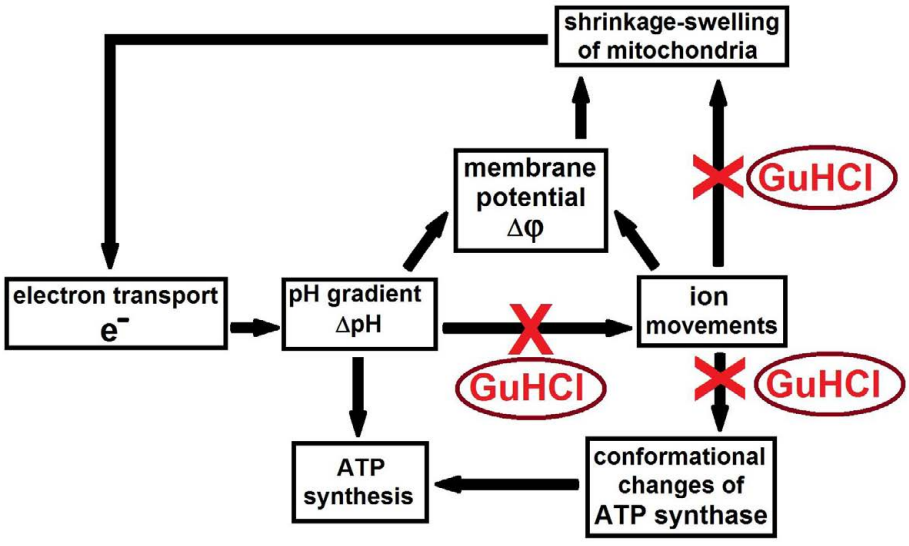
It is obvious that the guanidine breaks primarily the movement of calcium ions, and then associated with it a cyclic low-amplitude swelling-shrinkage. Mitochondrial pH and pH gradient decrease during cytosolic Ca^{2+} elevations and individual mitochondria undergo

spontaneous alkalization transients [24,25]. Calcium ions perform a regulatory role in the mitochondria and the cell. Correct Ca^{2+} uptake into the mitochondria is essential for mitochondrial oxidative phosphorylation, but excessive Ca^{2+} uptake leads to cell death [26]. Therefore, the balance between Ca^{2+} influx and efflux is fundamental for correct intracellular homeostasis and is extensively regulated by influx (through the mitochondrial Ca^{2+} uniporter) and efflux (through the Na^{+}/Ca^{2+} or H^{+}/Ca^{2+} exchanger) systems [27].

Increases in the free calcium ion concentration in the mitochondrial matrix activate mitochondrial dehydrogenases to stimulate oxidative phosphorylation [28] whereas Ca^{2+} elevations in the intermembrane space stimulate the uptake of substrates of oxidative phosphorylation [29,30]. The presence of calcium in the matrix promotes dehydrogenase, but inhibits the entry of calcium into the matrix of the mitochondria from the external environment, as well as transport of protons from the matrix into the outside environment in the presence of ADP. The accumulation of calcium in the inter-membrane space stimulates the uptake of oxidative phosphorylation substrates but due to the lack of calcium ions in the intermembrane space the absorption of oxidative phosphorylation substrates is inhibited and followed by respiration, despite the activation of dehydrogenases. With increasing concentrations of guanidine in the incubation medium mitochondrial respiration in State 4 will be suppressed depending on the concentration of guanidine. On the data of Bridges et al. [12] complex I and ATP synthase may be particularly susceptible to biguanide inhibition because of catalytic conformational mobility in their domain interfaces. However, in our experiments we used a succinate, which is a substrate of complex II. The site(s) at which biguanides bind to ATP synthase, and their inhibitory mechanism(s), are currently unknown [12]. Thus, first of all, we suggest, that the calcium ions are moved cyclically between the external environment, the matrix and intermembrane space activating various processes. A disruption of movement of calcium ions by various agents, including guanidine leads to disruption of ATP synthesis (Scheme).

Apparently, as we assume, alkilguanidinies unlike guanidine interact with the outer membrane of mitochondria binding to inner membrane in protonated state and directly violate the movement of ions. The same effect is characteristic for lipophilic compounds, such as 2,4-DNP. We believe that the uncoupling effect at low concentrations and inhibitory effects of respiration at high concentrations of 2,4-DNP [17] unlike alkilguanidinies are due to the violation of outer mitochondrial membrane permeability for potassium ions [18,31], which disrupts the movement of calcium ions. So, alkilguanidinies suppress predominantly respiration in State 3. Uncoupling and an inhibitory effect of valinomycin on mitochondria [18], also takes place by changing the permeability of the outer membrane to potassium ions, which disrupts, as we suggest, the movement of calcium ions.

Since the guanidine compounds having a negative effect on the phosphorylation ability of mitochondria would cause a more significant inhibition of mitochondrial activity in normal, healthy cells than in the cancer cells, as mitochondria in these cells are in a swollen state and less active [32]. It is known that [33] the cytosol of cancer cells has a more acidic pH. Taking into acount these facts, it can be assumed that the mechanism of cancer treatment will be in the pH changes by guanidine compounds. The selective penetration of the guanidine into the acidified cytosol of the cancer cells, might lead to the restoring of mitochondrial function. At the same time, the use of metabolically active forms of guanidine compounds can restore the function of mitochondria in cancer cells, curing such cells and cannot have any negative effects on the mitochondria of normal cells. For example, L-arginine (2-Amino-5-guanidinopentanoic acid) is such metabolically active guanidine compound. L-arginine plays an important role in numerous physiological processes [34] including nitrogen detoxification, inflammation, immunocompetence, growth hormone secretion, and insulin secretion, vascular dysfunction with aging and cardiovascular disease. Many modern diseases, including cancer, cardiovascular disease, diabetes, liver disease, arthritis, and neurodegenerative disease, are related to aging, and intensive research is being conducted to discover both the mechanisms of aging itself as well



Scheme: A schematic representation of the guanidine effect on the coupling of electron transport, ATP synthesis, movement of ions and low amplitude shrinkage-swelling of mitochondria. Red cross shows the place of action of guanidine.

as its relation to these conditions. It is assumed that an inflammation leads to aging through DNA damage, telomere dysfunction, cellular senescence, and oxidative stress.

Arginine is a stimulator of growth hormone [35], and the organism stops the synthesis of arginine in order to limit the growth after 28 years of age for a human (when a body stops growing and begins its aging).

With the shortage of arginine in the body, primarily synthesis of ATP by oxidative phosphorylation in the mitochondria is decreased, also ATP synthase may not be synthesized. Therefore, to compensate for the energy loss, the body shifts to a low-efficiency glycolysis [36] which is accompanied by the occurrence of type 2 diabetes. Then, changing the structure of the mitochondria occurs, which are in a swollen state [37]. We assume that such a structure promotes reactive oxygen species (ROS) production by cytochrome bc_1 [38] in excess, which in turn causes the first mutation of the mitochondrial DNA, and then, the nuclear DNA [39]. Hence, DNA mutations cause cancer.

Morever, telomeric repeat-binding factor 2 (TRF2) functions to protect telomeres and contains an N-terminal basic domain rich in arginines [40]. Arginine methylation regulates telomere length and stability. The repeated addition of the nitric oxide (NO) donor *S*-nitroso-penicillamine (L-arginine is a substrate for NO synthase, which catalyzes nitric oxide synthesis [36]) significantly reduced endothelial cells senescence and delayed age-dependent inhibition of telomerase activity [40]. Obesity or hypertension, the excess formation of reactive oxygen species (ROS) can lead to an accumulation of asymmetric dimethyl arginine (ADMA) [41,42]. This accumulation of ADMA competitively inhibits NO synthase (NOS), which decreases NO production. With limited L-arginine, NOS forms superoxide (O-), which causes vascular endothelial injury and further inflammation [43]. Rafikov et al. [44] found that in the absence of ADMA, endothelial nitric-oxide synthase (eNOS) translocation decreased mitochondrial oxygen consumption and superoxide production without altering cellular ATP level.

So, on the one hand, guanidine compounds play a key role as a means of storing and using metabolic energy (highenergy phosphoramidate bond), phosphoarginine in vertebrates and phosphocreatine in invertebrates [45]. Also, L-arginine is directly involved in the synthesis of ATP in accordance with the mechano-chemiosmotic model. On the other hand, L-arginine plays a role in numerous physiological processes. While the deficit of L-arginine in the body is the cause of many diseases, premature aging and reduce life expectancy.

In conclusion, it should be noted that aging of humans can be delayed after stopping of synthesis of endogenous arginine in the body (after about 28 years of age) by taking exogenous L-arginine (or L-arginine rich foods) and performing physical exercise, in which the possibility of prolonging human healthspan appears.

Acknowledgments

We thank Abdyukova Leila for helping in preparing the manuscript; Kasumova Victoriya for support; several anonymous reviewers for reading our manuscript and for giving us useful comments.

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