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Possible new mitochondrial mechanism of vitamin C anticancer activity

Posible nuevo mecanismo mitocondrial de la actividad anticancerígena de la vitamina C

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Abstract

The main feature of fast-growing malignant tumors is the Warburg-type metabolism, which is directly related to an extremely high quantity of hexokinase (HK) bound to the voltage-dependent anion channels (VDACs) in the mitochondrial outer membrane. Previously, we explained the Warburg type metabolism as a result of the electrical suppression of mitochondria due to the closure of free VDACs that are not bound to HK. Here, we propose a possible new mechanism of high-dose vitamin C (ascorbate) anticancer activity estimated with a simplified computational model. According to the proposed hypothesis and the model, ascorbate oxidation in mitochondria leads to the generation of the negative outer membrane potential (OMP) of opposite sign to the positive OMP generated by the VDAC-HK complexes in cancer cells. The model demonstrates that negative OMP generated by any mechanism, even of relatively low magnitudes, leads to the reopening of the electrically closed VDACs, thus reprogramming cell energy metabolism. According to the hypothesis, redox mediators, which increase the rate of ascorbate oxidation in mitochondria, should synergistically increase anticancer effects of high-dose ascorbate in accordance with experimental data recorded in the literature. The model shows that even small changes in the VDAC-voltage sensitivity and/or quantity of the VDAC-HK complexes, known to be caused by various physiological factors, might strongly influence the proposed mitochondrial mechanism of ascorbate anticancer activity.

Keywords: Vitamin C; Anticancer activity; Warburg metabolism; Mitochondria; VDAC; Mitochondrial outer membrane potential.

Resumen

La característica principal de los tumores malignos de crecimiento rápido es el metabolismo de tipo Warburg, directamente relacionado con una cantidad extremadamente alta de hexoquinasa (HQ) unida a los canales aniónicos dependientes de voltaje (VDAC) en la membrana mitocondrial externa. En un estudio anterior se explicó el metabolismo de tipo Warburg como resultado de la supresión eléctrica de las mitocondrias debido al cierre de los VDAC libres, no unidos a HQ. En este se propone un posible nuevo mecanismo de la actividad anticancerígena de altas dosis de vitamina C (ascorbato) estimado mediante un modelo computacional simplificado. Según la hipótesis propuesta y el modelo, la oxidación de ascorbato en las mitocondrias conduce a la generación del potencial negativo de la membrana externa (PME), de signo opuesto al PME positivo generado por los complejos VDAC-HQ en las células cancerosas. El modelo demuestra que el PME negativo, incluso de magnitudes relativamente bajas, generado mediante cualquier mecanismo, lleva a la reapertura de los VDAC cerrados eléctricamente, reprogramando así el metabolismo energético celular. Según la hipótesis, los mediadores redox, que aumentan la tasa de oxidación de ascorbato en las mitocondrias, deberían aumentar sinérgicamente los efectos anticancerígenos de las dosis altas de ascorbato en concordancia con los datos experimentales reportados en la literatura. El modelo muestra que, incluso pequeños cambios en la sensibilidad

al voltaje de los VDAC y/o de la cantidad de los complejos VDAC-HQ, causados por diversos factores fisiológicos como se sabe, podrían influir fuertemente en el mecanismo mitocondrial propuesto de la actividad anticancerígena del ascorbato.

Palabras clave: Vitamina C; Actividad anticáncer; Metabolismo de Warburg; Mitocondrias; VDAC; Potencial de membrana externa mitocondrial.

Introduction

More than 60 years ago, McCormick proposed the usage of vitamin C (ascorbate) to limit the cancer metastasis process (**McCormick,** 1954, 1959). In the 1970s, Cameron and Pauling supported this concept, using high intravenous doses of vitamin C, followed by high oral doses, for advanced cancer patients (**Cameron & Pauling,** 1976, 1978). The reported anticancer effects of vitamin C were not unequivocally convincing, and the use of high-dose ascorbate in the clinical treatment of human tumors is controversial even up to now (**Roa,** *et al.***,** 2020; **Bakalova,** *et al.***,** 2020; **Giansanti,** *et al.***,** 2021; **Zasowska-Nowak,** *et al.***,** 2021). On the other hand, anticancer properties of ascorbate have been demonstrated in experiments with various cancer cell lines *in vitro* (**Verrax,** *et al.***,** 2006; **Park,** 2013; **Ma,** *et al.***,** 2017; **Lv,** *et al.***,** 2018; **Semkova,** *et al.***,** 2020) and in animals (**Verrax,** *et al.***,** 2006; **Polireddy,** *et al.***,** 2017; **Xia,** *et al.***,** 2017; **Lv,** *et al.***,** 2018) including aggressive tumor xenografts in mice (**Chen,** *et al.,* 2008) and many other aggressive tumors (see for references **Polireddy,** *et al.,* 2017).

Anti-metastatic and other beneficial effects of intravenous high-dose ascorbate in patients with advanced stage tumors were shown in preclinical trials (**Polireddy,** *et al.***,** 2017; **Xia,** *et al.***,** 2017; **Bazzan,** *et al.***,** 2018; **Zhou,** *et al.***,** 2020; **Zasowska-Nowak,** *et al.***,** 2021). It is very important to highlight that the high intravenous doses of ascorbate needed for anticancer effects allow plasma concentrations up to two orders of magnitude higher than concentrations achievable at the highest tolerated oral doses (**Zasowska-Nowak,** *et al.***,** 2021).

The mechanism of the ascorbate anticancer activity is not yet clear and appears to have multiple etiologies (**Park,** 2013; **Ma,** *et al.***,** 2017; **Polireddy,** *et al.***,** 2017; **Lv,** *et al.***,** 2018; **Bakalova,** *et al.***,** 2020). At least one of them might relate to the ascorbate oxidation in mitochondria as these organelles play a crucial role in switching cell energy metabolism from the preferentially aerobic oxidative phosphorylation in normal cells to the aerobic glycolysis observed in many malignant tissues, a process known as the Warburg type metabolism (**Nakashima,** *et al.***,** 1988; **Lemeshko,** 2002; **Marín-Hernández,** *et al.***,** 2006; **Maldonado,** *et al.***,** 2013). Previously, we explained such metabolic switching as a result of the metabolically-dependent generation of OMP by the VDAC-HK complexes (**Lemeshko,** 2014, 2015, 2017) functioning as a biological battery (**Lemeshko,** 2017, 2021). The generated OMP, positive in the mitochondrial intermembrane space (IMS), restricts the mitochondrial outer membrane (MOM) permeability to negatively charged metabolites, mainly for ATP (**Rostovtseva & Colombini,** 1997; **Colombini,** 2016).

Ascorbate is known to be oxidized very slowly in intact mitochondria, which allows assuming that MOM is impermeable to this anion (**Lehninger,** *et al.***,** 1954; **Valenti,** *et al.***,** 2014). In contrast, we have suggested that metabolically-dependent OMP of relatively high magnitude might be generated during ascorbate oxidation (**Lemeshko,** 2018, 2021) leading to the electrical closure of free VDACs in MOM and decreasing MOM permeability to this anion (negative feedback control). We also assumed (**Lemeshko,** 2021) that this ascorbate-dependent tendency to generate OMP (**Figure 1A**) negative in IMS might modulate the OMP generated in the mitochondria of cancer cells, controlling cell energy metabolism.

Here we used an essentially simplified computational model to evaluate the proposed hypothesis and demonstrate that the magnitude of ascorbate-dependent OMP is sufficiently high to decrease significantly the positive OMP generated by the VDAC-HK complexes in mitochondria of cancer cells ($\Delta\psi_o$ in **figure 1B**), thus reprogramming the type of cell energy metabolism and decreasing cell death resistance. This mechanism may be modulated
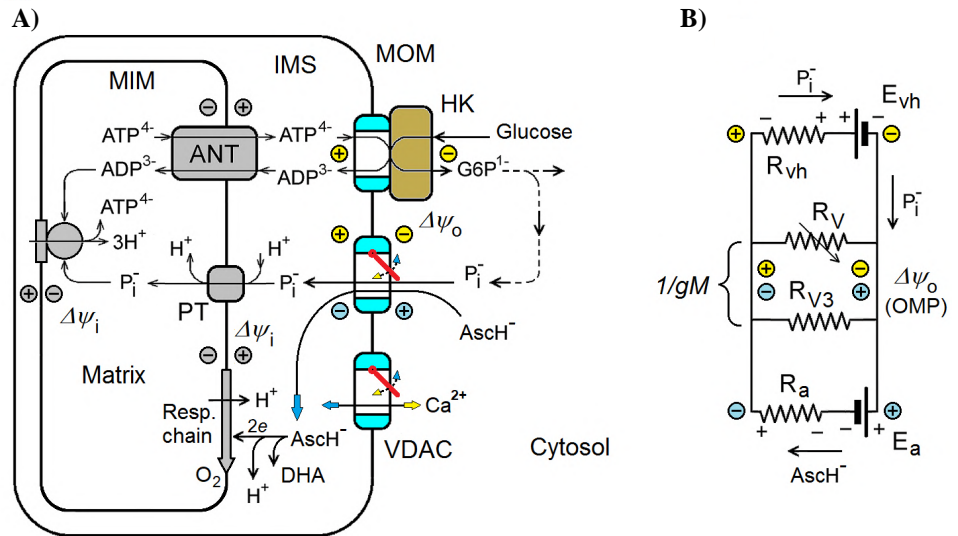
Figure 1. Modulation of the MOM permeability in cancer cells by the mitochondrial oxidation of ascorbate, AscH⁻. **A.** Ascorbate oxidation allows decreasing the magnitude of the positive OMP generated by the VDAC-HK complexes leading to the reopening of free VDACs in MOM, thus reprogramming the cancer type of cell energy metabolism and decreasing electrical, OMP-dependent Ca^{2+} extrusion from mitochondria. **B.** Equivalent electrical circuit of model **A** where the VDAC-HK complexes function as a biological battery (E_{hv}) with the internal resistance R_{vh} maintaining the circulation of inorganic phosphate through MOM and generating the positive OMP. Steady-state transportation and oxidation of ascorbate in IMS have a tendency to generate the opposite, negative OMP, thus decreasing the magnitude of the final positive OMP ($\Delta\psi_o$) and increasing MOM permeability. MIM: Mitochondrial inner membrane; ANT: Adenine nucleotide translocator; PT: Inorganic phosphate transporter; DHA: Dehydroascorbate; E_a: Ascorbate Nernstian potential; R_v: Resistance of free VDACs in MOM (VDAC1 and VDAC2); R_{V3}: Resistance of free VDAC3 in MOM; R_a: Internal resistance of the battery E_a. gM: MOM permeability

by many physiological factors influencing the VDAC-voltage sensitivity, VDAC-HK interactions, and VDAC permeability. All these aspects seem to be useful for developing new strategies for anticancer combination therapy.

Materials and methods

Model for the generation of the mitochondrial outer membrane potential by VDAC-hexokinase complexes

According to the model shown in **figure 1A**, some percentage of VDACs in MOM forms VDAC-HK complexes functioning as a biological battery (E_{vh} in **Figure 1B**) (**Lemeshko,** 2017, 2018, 2021). Part of the battery voltage drops on the MOM ($\Delta\psi_o$) and another part is applied to the internal resistance R_{vh} of the battery (**Figure 1B**). R_{vh} is inversely proportional to the fraction of VDACs bound to HK, N_{vh}. According to the model, 10% of all VDACs in MOM are represented by the low voltage-sensitive porin isoform VDAC3, N_3, which does not bind to HK. The major fraction of free VDACs in MOM is represented by the voltage-sensitive VDAC isoforms, N_v, such as VDAC1 and VDAC2. The quantity of all VDACs in MOM was normalized to 100:

$$100 = N_3 + N_{vh} + N_v, \qquad (1)$$

where $N_3 = 10$ and N_{vh} was taken for estimations equal to 4.0 or 3.6 relative units (r.u.).

The conductance of all MOM fractions of VDACs in their completely open states was also normalized to 100 r.u.:

$$100 = g3 + gV_0 + gH, \qquad (2)$$

where gV_0 is the conductance of the VDAC fraction N_v at zero membrane potential, gH is the conductance of the fraction N_{vh} of completely open VDACs, yet not restricted by the HK binding (numerically, $gH = N_{vh}$), $g3 = 10$ r.u., assuming that the permeability of the VDAC3 fraction is completely voltage-insensitive, although the model also allows consideration of the redox-dependent modulation of the VDAC3 voltage sensitivity (**De Pinto,** *et al.,* 2016).

The conductance gVH of the fraction N_{vh} of VDACs bound to HK in MOM was considered to depend on the glucose concentration in the cytosol, $[Gl]_o$, as (**Lemeshko,** 2017):

$$gVH = \frac{N_{vh} \cdot [Gl]_o}{K_{m,G} + [Gl]_o}, \qquad (3)$$

where the Michaelis–Menten constant $K_{m,G} = 0.1$ mM glucose. In other words, gVH also represents mitochondrial hexokinase activity in relative units considering N_{vh} as the maximum HK activity. The MOM permeability gV through the fraction N_v of free voltage-sensitive VDACs was considered to be equal for both the inorganic phosphate P_i^{-1} and the ascorbate anion AscH⁻, and expressed as a bell-shaped function of OMP (**Lemeshko,** 2014, 2017):

$$gV = gV_0 \cdot g_C + gV_0 \cdot (1 - g_C) \cdot \exp{(-S \cdot OMP^2)}, \quad (4)$$

where $g_C = 0.2$ is taken as the relative VDAC permeability in the closed state and S is the voltage sensitivity parameter. The resulting normalized "VDAC's conductance-OMP" functions at the voltage sensitivity parameters $S = 40$ V⁻¹ and $S = 36$ V⁻¹ are shown in **figure 2** (*a* and *b*, respectively).

The total MOM permeability, gM, for P_i^{-1} or the ascorbate anion AscH⁻ was presented as:

$$gM = g3 + gV. \qquad (5)$$

The voltage generated by the VDAC-HK complexes (E_{vh} in **figure 1B**) depends on the Gibbs free energy of the mitochondrial HK reaction (**Lemeshko,** 2014, 2017, 2021) as:

$$E_{vh} = -\left(\frac{\Delta G^o_{HK}}{F} + \frac{RT}{F} \ln \frac{[ADP]_i \ [G6P^{1-}]_o}{[ATP]_i \ [Gl]_o}\right), \qquad (6)$$

where $\Delta G^o_{HK} = -16.7$ kJ/mol is the standard Gibbs free energy of the hexokinase reaction, R is the universal gas constant, F is the Faraday constant, and $T = 310\ K$ is the normal body temperature. $[ADP]_i$ and $[ATP]_i$ are IMS concentrations of ADP and ATP, respectively, $[Gl]_o$ and $[G6P]_o$ are the concentrations of glucose and glucose-6-phosphate, respectively, in the cytosol. For all estimations, it was taken that the ratio $[ATP]_i/[ADP]_i = 100$ and $[G6P]_o = 0.1$ mM.
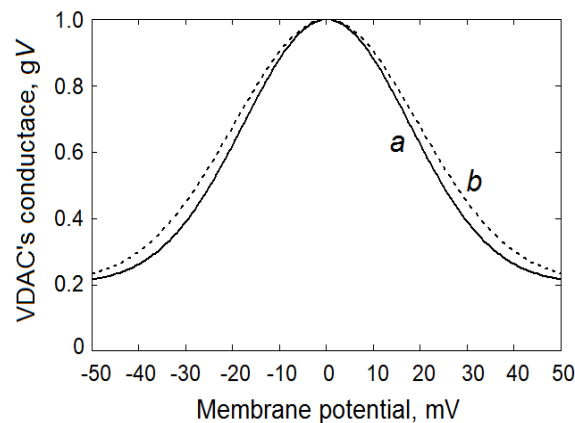
Figure 2. VDAC voltage-gating properties used in the computational model: *a)* at the VDAC voltage sensitivity parameter $S = 40$ V⁻¹; *b)* at $S = 36$ V⁻¹ (equation 4)

In the absence of ascorbate (*i.e.*, at $E_a = 0$), the equivalent electrical circuit shown in **figure 1B** may be described according to Ohm's law as:

$$E_{vh} = \frac{I_h}{gVH} + \frac{I_h}{gM}, \qquad (7)$$

where I_h is the hexokinase-mediated current (in dimensionless units).

According to equation 7, the OMP generated by the VDAC-HK complexes ($\Delta\psi_o$ in **figure 1B**) may be presented as the voltage drop on the MOM resistance, $1/gM$:

$$OMP = \frac{I_h}{gM}. \qquad (8)$$

The units of OMP (equation 8), as well as of E_{vh} (equations 6 and 7), come in volts.

If a negative MOM potential f is generated by some mechanism simultaneously with the functioning of the VDAC-HK complexes, the resulting OMP may be presented as:

$$OMP = \frac{I_h}{gM} + f, \qquad (9)$$

where f (of negative sign) is given in volts.

The system of equations 1-9 may be solved at given parameters $g3$, gH and f, calculating gV, gM and OMP as functions of glucose concentration in the cytosol.

Model of ascorbate-dependent generation of the outer membrane potential in intact mitochondria

The steady state rate of ascorbate oxidation in intact mitochondria, v_A, should be equal to the Nernst-Planck flux of this anion through MOM:

$$v_A = gM \cdot \frac{F}{RT} \cdot OMP_A \cdot \frac{[A]_o - [A]_i \cdot \exp\left(-\frac{F \cdot}{RT} \cdot OMP_A\right)}{1 - \exp\left(-\frac{F \cdot}{RT} \cdot OMP_A\right)}, \qquad (10)$$

where $[A]_o$ and $[A]_i$ are the ascorbate concentrations in the cytosol and IMS, respectively, and OMP_A is the MOM potential generated by the ascorbate flux across the membrane.

The rate v_A of ascorbate oxidation by cytochrome c in IMS is presented by the Michaelis–Menten kinetics,

$$v_A = \frac{V_m \cdot [A]_i}{K_{m,A} + [A]_i}, \qquad (11)$$

where V_m is the maximum rate of oxidation in the same relative units (r.u.) as the hexokinase activity, gVH, the constant $K_{m,A} = 66$ mM ascorbate (**Yonetani,** 1960), and $[A]_i$ is the concentration of ascorbate in IMS. For calculations, V_m was taken at 0, 4 or 40 r.u. and $N_{vh} = 4$ r.u. OMP_A was estimated by solving the system of equations 1, 2, 4, 5, 10 and 11 as a function of ascorbate concentrations.

The software Mathcad Professional was used to perform all mathematical calculations.

Results

The MOM VDAC-HK complexes function as a biological battery (**Lemeshko,** 2017, 2018, 2021) due to an active movement of the inorganic phosphate anion through MOM (**Figure 1A**) from ATP^{4-} in IMS to glucose in the cytosol forming glucose-6-phosphate. This movement depends on the free energy of the hexokinase reaction. Inorganic phosphate then returns into IMS, thus closing the electrical circuit to recover ATP in the mitochondrial matrix. In this case, OMP generation may be presented as a part of E_{vh}, *i.e.*, as the voltage drop on the equivalent resistances *1/gM* of MOM (**Lemeshko,** 2014, 2021) (**Figure 1B**).

According to our estimations using the computational model, the magnitude of OMP, positive in IMS, depends on the concentration of glucose (**Figure 3,** curve *a*). With a 10 % decrease in the quantity of VDAC-HK complexes in MOM, from 4 r.u. (**Figure 3A,** curve *a*) to 3.6 r.u. of all VDACs in MOM (**Figure 3B,** curve *a*), the critical glucose concentration at which OMP begins to increase sharply shifted from 2 mM (at 4 r.u.) to 6 mM (at 3.6 r.u.), approximately.
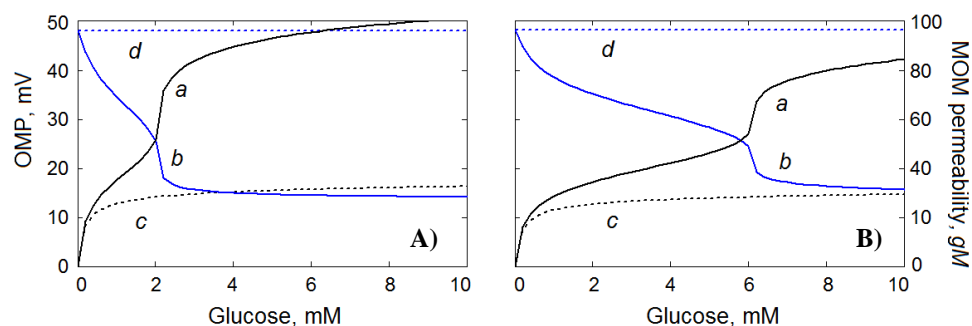
Figure 3. Generation of OMP (**a, c**) by the VDAC-HK complexes in mitochondria and the MOM permeability to inorganic phosphate and ascorbate (**b, d**) as functions of glucose concentration. The fraction N_{vh} of the VDAC-HK complexes - 4.0 r.u. (**A**) or 3.6 r.u. (**B**). The VDAC voltage sensitivity parameter $S = 40$ V^{-1} (**a, b**) or $S = 0$ V^{-1} (**c, d**) (equation 4). Calculations were performed according to section 2.1.

The model also demonstrated a remarkable OMP-dependent decrease in the MOM permeability, *gM* (**Figure 3**, curve *b*). In contrast, at the zero VDAC voltage sensitivity parameter, $S = 0$ V^{-1} (equation 4), the generated OMP has relatively low magnitudes (**Figure 3**, curve *c*) without its somewhat essential influence on the MOM permeability (**Figure 3**, curve *d*).

The model also demonstrated that a negative MOM potential, *f*, generated by any mechanism significantly influences the resulting OMP (equation 9) generated during the simultaneous functioning of the VDAC-HK complexes. The potential *f* of the magnitudes higher than $f = -6.5$ mV for the case of 4.0 r.u. of the VDAC-HK complexes (**Figure 4A**) and the potential higher than $f = -2.0$ mV for the case of 3.6 r.u. of the VDAC-HK complexes (**Figure 4C**), completely prevent a sharp glucose-dependent increase in OMP, at least in the range up to 10 mM glucose concentration, thus allowing higher MOM permeability, *gM* (**Figure 4**, **B** and **D**, respectively).

Similar data to the case of a 10 % decrease in the quantity of the VDAC-HK complexes in MOM (**Figure 4**, **C** and **D**) were also obtained by a 10 % decrease in the VDAC voltage sensitivity parameter, from S = 40 V^{-1} to S = 36 V^{-1} (**Figure 4**, **E** and **F**). Besides, a simultaneous 10% decrease in the quantity of the VDAC-HK complexes and the VDAC voltage sensitivity completely prevented any sharp glucose-dependent increase in OMP (data not shown).

In other words, the data presented in **figure 4** demonstrate that a decrease in the quantity of hexokinase attached to mitochondria and/or a decrease in the voltage sensitivity of the VDAC gating mechanism, strongly influence glucose-dependent VDAC-HK-mediated regulation of the MOM permeability.

To evaluate the magnitude of the MOM potential generated only under ascorbate oxidation in intact mitochondria, OMP$_A$, the estimations were performed assuming completely silenced VDAC-HK complexes in MOM ($N_{vh} = 4$ r.u.), as it would be at zero concentration of glucose. The model demonstrated the generation of relatively high OMP$_A$ negative in IMS at different maximum rates, V_m, of ascorbate oxidation by cytochrome *c* in IMS and at given concentrations of ascorbate ($[A]_o$ and $[A]_i$) (**Figure 5**).

First of all, if we assume the rate $V_m = 0$, *i.e.* the thermodynamic equilibrium, we have Nernstian potential at given values of $[A]_o$ and $[A]_i$ (**Figure 5A**). At steady-state with $V_m = 4$ r.u. and the VDAC voltage sensitivity parameter $S = 40$ V^{-1}, the model demonstrated OMP$_A$ of lower magnitudes (**Figure 5B**) than Nernstian potentials, as well as a more significant decrease in OMP$_A$ at higher V_m, for example at $V_m = 40$ r.u. (**Figure 5C**). A decrease in OMP$_A$ at steady states is a result of the voltage drop (part of the Nernstian potential) on the internal resistance R$_a$ of the ascorbate battery E_a (**Figure 1B**). In the case of $V_m = 40$ r.u., if we additionally decrease the voltage sensitivity parameter from $S = 40$ V^{-1} to $S = 0$ V^{-1},
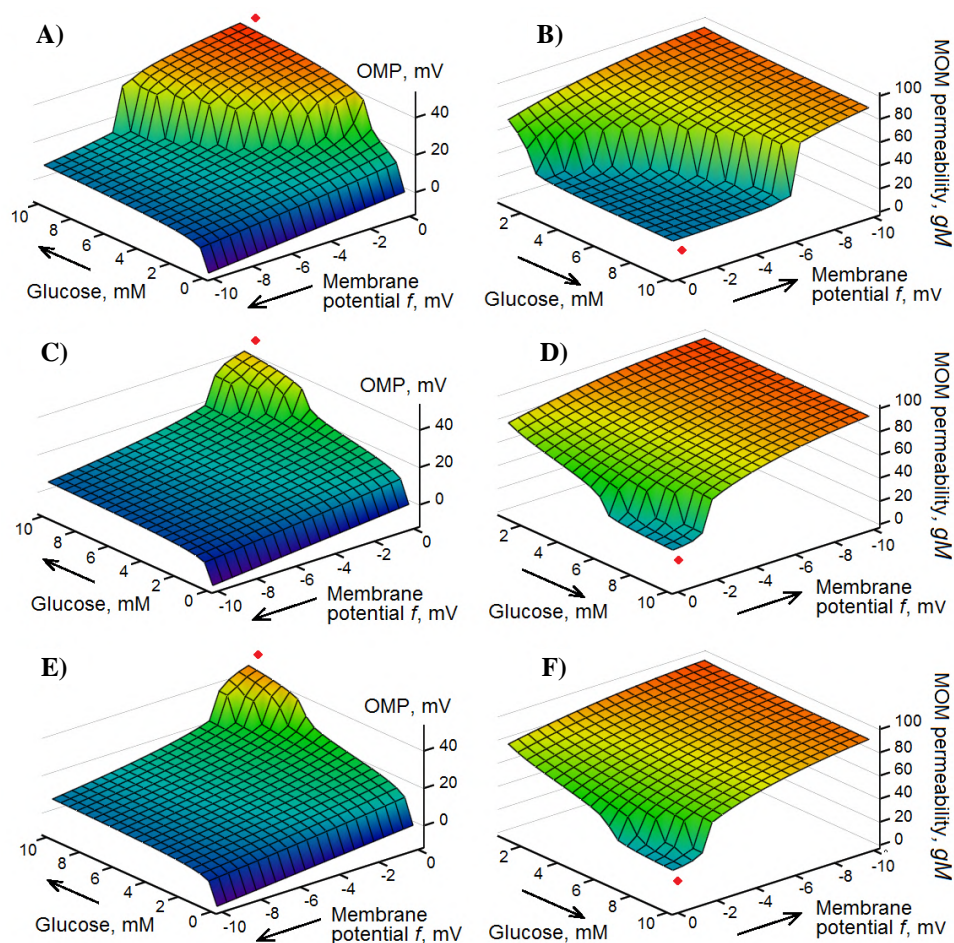
Figure 4. Influence of the negative membrane potential f generated by any mechanism simultaneously with the generation of positive OMP by the VDAC-HK complexes in mitochondria on the resulting OMP (equation 9) (**A, C, E**) and the MOM permeability gM (equation 4) (**B, D, F**). The fraction N_{vh} of the VDAC-HK complexes – 4.0 r.u. (**A, B**) or 3.6 r.u. (**C, D**) at the VDAC voltage sensitivity parameter $S = 40$ V^{-1}. **E, F:** $N_{vh} = 4.0$ r.u. and $S = 36$ V^{-1}

the model demonstrates significantly increased magnitudes of OMP$_A$, in the same range of given concentrations of ascorbate (**Figure 5D**) due to a lower voltage drop on the resistance R_a (**Figure 1B**).

In general, the model demonstrates that the membrane potential of relatively small magnitudes, negative in IMS (f in **figure 4**) and generated by any mechanism, might modulate the positive OMP generated by the VDAC-HK complexes and the MOM permeability. Additionally, it was clear that the ascorbate oxidation in intact mitochondria functions is one of these mechanisms and it has the tendency to generate the negative membrane potential.

Discussion

As a natural product, ascorbate is of great interest for its possible clinical use in tumor treatments, at least in combination with other methods of chemotherapy. Besides, it does not demonstrate noticeable toxicity against normal tissues, even at high doses, having, instead, various positive effects for the treatment of advanced-stage cancer (**Polireddy, *et al.***, 2017; **Xia, *et al.***, 2017; **Bazzan, *et al.***, 2018; **Zhou, *et al.***, 2020; **Zasowska-Nowak, *et al.***, 2021). According to the literature data (**Park**, 2013; **Ma, *et al.***, 2017; **Polireddy, *et***
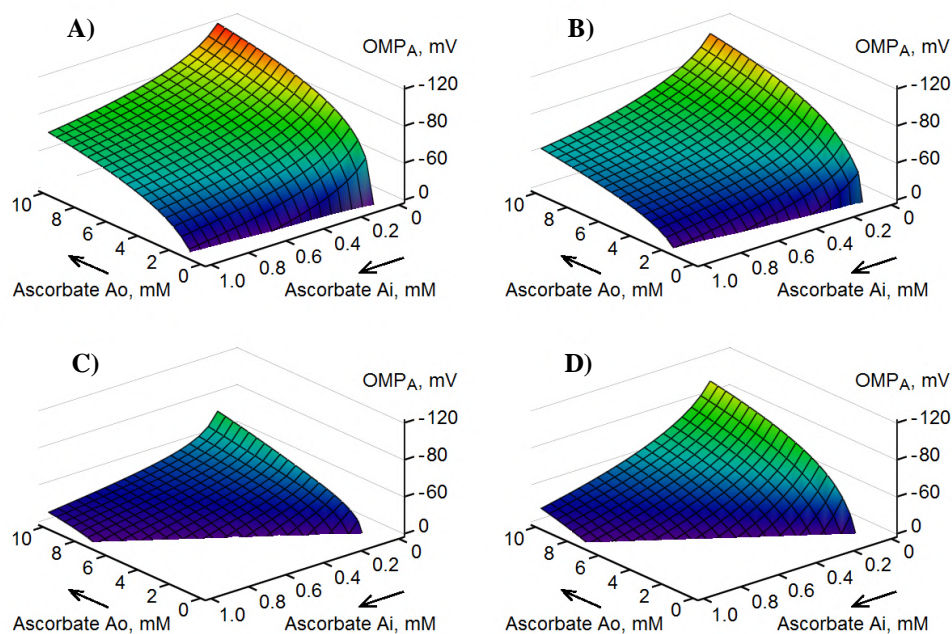
Figure 5. Generation of OMP_A during ascorbate oxidation in intact mitochondria at $V_m = 0$ r.u. (**A**), $V_m = 4$ r.u. (**B**), $V_m = 40$ r.u. (**C, D**), $S = 40$ V^{-1} (**A-C**), or $S = 0$ V^{-1} (**D**). Estimations were performed at $N_{vh} = 4$ r.u. V_m: Maximum rate of ascorbate oxidation in IMS.

al., 2017; **Lv, et al.,** 2018; **Bakalova, et al.,** 2020), there seem to be multiple mechanisms of the ascorbate anticancer activity that are not entirely understood. It means that many factors might modulate its efficiency in the treatment of malignant tissues (**Noto, et al.,** 1989; **Verrax, et al.,** 2006; **Gogvadze, et al.,** 2008; **González, et al.,** 2012; **van Gorkom, et al.,** 2019). Here we propose a new mechanism of ascorbate anticancer activity focusing our attention on the great difference in the energy metabolism of cancer versus normal cells (**Nakashima, et al.,** 1988; **Lemeshko,** 2002; **Marín-Hernández, et al.,** 2006; **Gogvadze, et al.,** 2008; **González, et al.,** 2012; **Maldonado, et al.,** 2013).

We had already explained the Warburg type metabolism of cancer cells as a result of the electrical suppression of mitochondria by the metabolically-dependent OMP generated by the VDAC-HK complexes in MOM (**Lemeshko,** 2002, 2014, 2015, 2021). According to the computational model presented in this work (**Figure 1**), the critical glucose concentration needed for the generation of relatively high-magnitude OMP strongly depends on the quantity of these complexes in MOM (**Figure 3**). Our estimations demonstrate that the superposition of a relatively small negative OMP (*f*) generated by any mechanism and the positive OMP generated by the VDAC-HK complexes strongly decreases the magnitude of the resulting OMP. This OMP decrease leads to the reopening of the electrically closed free VDACs in MOM, thus increasing MOM permeability (**Figure 4**).

The model demonstrated that the effect of the mentioned negative potential *f* on the MOM permeability strongly depends on the quantity of the VDAC-HK complexes. A mere 10% decrease in the quantity of the VDAC-HK leads to a significant decrease in the glucose-dependent OMP (**Figure 4C**), thus increasing MOM permeability (**Figure 4D**). In this respect, many factors that can prevent the formation of the VDAC-HK complexes in MOM, or induce their dissociation, have been reported to demonstrate pro-apoptotic and anticancer activities (**Galluzzi, et al.,** 2008; **Camara, et al.,** 2017; **Magrì, et al.,** 2018; **Shteinfer-Kuzmine, et al.,** 2018; **Ciscato, et al.,** 2020). The mechanism of such pro-apoptotic and anticancer activities might involve the mentioned reopening of the electrically closed VDACs in the mitochondria of cancer cells.

The model also demonstrated that the modulation by a negative membrane potential *f* of OMP generated by the VDAC-HK complexes strongly depends on VDAC voltage sensitivity allowing the reopening of the electrically closed VDACs. A decrease of only 10% in the voltage sensitivity parameter *S* leads to a strong decrease in OMP, thus increasing MOM permeability (**Figure 4**, **E** and **F**). In this respect, VDAC voltage sensitivity may be essentially modulated by many factors (**Rostovtseva,** *et al.*, 2020, 2021) including cytosolic NADH and NADPH (**Lee & Colombini,** 1996).

Some cytosolic proteins, such as tubulin and alpha-synuclein, have been demonstrated to cork up free VDACs in MOM assuming this process is strongly OMP-dependent (**Rostovtseva,** *et al.*, 2021). VDAC corking up, in turn, should also modulate OMP generation (**Lemeshko**, 2017, 2018, 2021).

As we assumed earlier (**Lemeshko,** 2018, 2021), ascorbate oxidation in intact mitochondria might generate OMP negative in IMS (**Figure 1**) thus explaining a very low rate of its oxidation in these organelles (**Lemeshko,** *et al.*, 2006; **Valenti,** *et al.*, 2014). The ascorbate anion is almost three times smaller than ATP^{4-}, although ATP^{4-} is permeable through the open VDAC (**Rostovtseva & Colombini,** 1997; **Colombini,** 2016; **Rostovtseva,** *et al.*, 2021). That is why there are no reasons to believe that MOM is impermeable to the ascorbate anion as assumed earlier (**Lehninger,** *et al.*, 1954; **Valenti** *et al.*, 2014). To the best of our knowledge, no experimental data regarding the VDAC permeability to ascorbate has been registered yet.

On the other hand, if ascorbate oxidation in mitochondria has the tendency to generate OMP negative in IMS (**Figure 5**), the anticancer effect of high-dose ascorbate might relate to the modulation of the positive OMP generated by the VDAC-HK complexes. The effect might be similar to that shown in **figure 4** for a negative potential *f* generated by any mechanism. Moreover, the positive VDAC-HK-dependent OMP might strongly increase ascorbate oxidation in the mitochondria of cancer cells by increasing steady-state-concentrated ascorbate in IMS. This conclusion is consistent with the experimental observations of vitamin C deficiency in cancer patients and malignant tissues (**Van Gorkom,** *et al.*, 2019; **Blaszczak,** *et al.*, 2019).

It has been reported that electrically-closed VDACs strongly restrict the release of superoxide anions from mitochondria (**Han,** *et al.*, 2003) meaning that if the VDAC-HK complexes in MOM generate OMP, there should be a relatively high concentration of superoxide anions maintained in the IMS of the mitochondria of cancer cells, as well as of ascorbyl-free radical anions due both to the electrical closure of free VDACs in MOM and to the generated OMP positive in IMS.

Interestingly, the protective, anti-apoptotic effect of mitochondrial HK in cancer cells has been considered to be a result not only of the VDAC's binding to MOM (two orders of magnitude higher than in normal cells) but also of the functioning of HK bound to the mitochondria (**Sun,** *et al.*, 2008), both important for the metabolically-dependent generation of OMP (**Lemeshko,** 2014, 2021). For example, the positive VDAC-HK-dependent OMP of 30 mV should maintain Ca^{2+} concentration in IMS one order of magnitude less than in the cytosol thus strongly preventing the Ca^{2+} overload of the mitochondria (**Figure 1A**). This is consistent with the fact that malignant tissues are highly resistant to cell death (**Han,** *et al.*, 2003; **Gogvadze,** *et al.*, 2008; **Sun,** *et al.*, 2008). The positive OMP generated by the VDAC-HK complexes may be decreased by applying the additional negative potential (*f*) (**Figure 4**) seemingly generated during ascorbate oxidation in the mitochondria of cancer cells (**Figure 5**). It should also facilitate Ca^{2+} entrance into IMS from the cytosol (**Figure 1A**) finally leading to Ca^{2+} overload of the mitochondrial matrix, even though the permeability to Ca^{2+} ions of VDAC in its electrically closed state is higher than in the completely open state (**Tan & Colombini,** 2007).

One of the main mechanisms suggested to explain ascorbate's anti-cancer properties is the ascorbate-induced ROS overproduction in cancer cells (**Verrax,** *et al.*, 2006; **Ma,** *et al.*, 2017; **Xia,** *et al.*, 2017; **Lv,** *et al.*, 2018; **Semkova,** *et al.*, 2020; **Zhou,** *et al.*, 2020) including ascorbyl radical generation that facilitates external NADH oxidation in mitochondria

and changes the cytosolic NADH/NAD$^+$ redox state (**Bakalova, *et al*.,** 2020). Extremely high production of mitochondrial superoxide in cancer cells during ascorbate oxidation facilitated by menadione has also been reported (**Semkova, *et al*.,** 2020). Increased ROS production in combination with Ca^{2+} overload of mitochondria should increase the probability of Ca^{2+}-ROS induced mitochondrial permeability transition (**Grimm & Brdiczka,** 2007; **Bernardi,** 2013; **Lemeshko,** 2018, 2021). Taking into account ROS-dependent mechanisms of high-dose ascorbate toxicity for malignant tissues (**Kang, *et al*.,** 2003; **Carosio, *et al*.,** 2007; **González, *et al*.,** 2010; **Xia, *et al*.,** 2017; **Zhou, *et al*.,** 2020), the possibility of generating the negative membrane potential during ascorbate oxidation (OMP$_A$ in **figure 5**) and its influence of the positive OMP generated by the VDAC-HK complexes in the mitochondria of cancer cells (**Figure 4**), the main mechanism of ascorbate anticancer activity seems to be combined OMP-Ca^{2+}-ROS-dependent.

The anti-cancer activity of ascorbate has been reported in the treatment of many aggressive tumors, such as pancreatic cancer, glioblastoma, ovarian cancer, prostate cancer, hepatoma, colon cancer, sarcoma, leukemia, mesothelioma, breast cancer, and neuroblastoma (see for references **Polireddy, *et al*.,** 2017). Interestingly, activities of the respiratory chain complexes in glioblastoma (WHO grade IV), for example, are significantly decreased in comparison with a normal brain, especially of complexes III (by 81%) and IV (by 84%) (**Feichtinger, *et al*.,** 2014). According to these data, the concentration of reduced cytochrome *c* in brain tumors and breast cancers is significantly higher than in normal human tissues (**Abramczyk, *et al*.,** 2021). The mentioned decrease in the respiratory chain complexes in the mitochondria of cancer cells might be an adaptive consequence of the Warburg-type metabolism, explained earlier as a result of electrical suppression of mitochondria by OMP generated by the abundant VDAC-HK complexes (**Lemeshko,** 2015, 2021).

In this respect, ascorbate is known to be oxidized in mitochondria with the participation of the cytochrome *c* and complex IV of the respiratory chain. The parameter V_m used in the model we present here (**Figure 5**) directly relates to complex IV and cytochrome *c* concentrations in IMS. The suggested model of the anti-cancer activity of ascorbate may be further developed in the future taking into account the mentioned changes in the respiratory chain, as well as the mutual interactions of the steady-state fluxes of inorganic phosphate and ascorbate across MOM.

It is well known that the redox mediators, such as menadione, PMS, TMPD, etc., strongly increase the rate of reduction of free cytochrome *c* by ascorbate, as well as the ascorbate oxidation in intact mitochondria (**Sanadi,** 1964, **Hill & Nicholls,** 1980; **Nicholls, *et al*.,** 1980; **Valenti, *et al*.,** 2014). This may explain the reported synergistic anti-cancer effects of ascorbate-menadione (**Noto, *et al*.,** 1989; **Gilloteaux, *et al*.,** 2006; **Verrax, *et al*.,** 2006; **Semkova, *et al*.,** 2020) and of the ascorbate use together with other redox mediators (**Tomasetti, *et al*.,** 2015; **Hua, *et al*.,** 2019).

In general, the results of the computational analysis of the suggested mechanism of ascorbate anti-cancer activity show that ascorbate oxidation in mitochondria has a tendency to generate negative OMP. This might modulate OMP generated by the VDAC-HK complexes in cancer cells leading to the reprogramming of the cell energy metabolism. This mechanism seems to be useful for developing novel and/or improving existing strategies of anti-cancer combination therapy (**Ngo, *et al*.,** 2019; **Semkova, *et al*.,** 2020), as well as for explaining in more detail various experimental phenomena in this respect.

Conclusion

Despite the long controversial history of ascorbate's anti-cancer properties, the mechanism of its selective toxicity at high doses for malignant tissues remains unclear and it seems to involve multiple processes and targets. Among possible targets underlying ascorbate anticancer effects, mitochondria might play a crucial role (**Gogvadze, *et al*.,** 2008; **González, *et al*.,** 2010, 2012; **Hua, *et al*.,** 2019; **Semkova, *et al*.,** 2020). In the case of

fast-growing tumor cells, the quantity of hexokinase attached to MOM is up to two orders of magnitude higher than in normal cells (**Marín-Hernández, et al.,** 2006). This allowed us to consider the VDAC-HK complexes in MOM as a biological battery (**Lemeshko,** 2017, 2018, 2021), generating high-magnitude OMP (positive in IMS) and switching cell energy metabolism to the Warburg type due to an electrical suppression of mitochondria (**Lemeshko,** 2015, 2021). On the other hand, the rate of ascorbate oxidation in isolated intact mitochondria of normal tissues is known to be extremely low, which seems to be caused by the generation of the negative OMP. The simplified computational model presented here demonstrated that a negative OMP of even low magnitudes generated by any mechanism might essentially decrease the magnitude of the positive OMP generated by the VDAC-HK complexes in cancer cells (**Figure 1A**) reprogramming the cell energy mechanism. This should also lead to the Ca^{2+}-ROS mitochondrial permeability transition, the cytochrome *c* release from mitochondria, and cell death. Besides, since the suggested primary mechanism of selective toxicity of ascorbate for cancer cells strongly depends on the presence of redox mediators and on factors influencing the VDAC-HK interactions, VDAC's voltage sensitivity, and VDAC's corking up factors (**Lemeshko,** 2017), the suggested mechanism might be useful for the development of new experimental approaches and novel anti-cancer combination therapies.

Acknowledgments

Conflicts of interest

The author declares no conflicts of interest

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