Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2026**; 31(9): 55451
https://doi.org/10.31083/FBL55451

Original Research

The Protective Effect of Venetoclax on the Calcium-Induced Mitochondrial Pore and ROS Production as a New Possible Factor Contributing to Drug Resistance

Alexey G. Kruglov[1], Tatiana A. Fedotcheva[2], Nadezhda I. Fedotcheva[1,*]

[1]Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, 142290 Pushchino, Moscow Region, Russia
[2]Science Research Laboratory of Molecular Pharmacology, Medical Biological Faculty, Pirogov Russian National Research Medical University, Ministry of Health of the Russian Federation, 117997 Moscow, Russia
*Correspondence: nfedotcheva@mail.ru (Nadezhda I. Fedotcheva)
Academic Editor: Jordi Sastre-Serra
Submitted: 30 June 2026 Revised: 13 August 2026 Accepted: 19 August 2026 Published: 20 September 2026

Abstract

Objective: Recent data show that there are direct relationships between mitochondrial activity, cancer progression, and chemoresistance. Venetoclax (Ven) is a mitochondria-targeted chemotherapeutic drug, which facilitates apoptosis by selectively inhibiting the antiapoptotic protein Bcl-2. Ven exhibits a dual effect, affecting not only mitochondria but also the cell drug resistance system. There is also evidence indicating that cancer cells develop resistance to Ven, which is mediated by the upregulation of antiapoptotic proteins, simultaneous activation of multiple signaling pathways, an alteration in the cellular and mitochondrial metabolism, and other defensive mechanisms. The influence of Ven on the opening of the mitochondrial permeability transition pore (mPTP), a key event in the induction of mitochondria-dependent cell death, has not been previously studied. **Methods:** The effect of Ven on calcium-induced mPTP opening, respiration, and reactive oxygen species (ROS) production was examined in isolated rat liver mitochondria, using cation-selective electrodes, as well as fluorescent and chemiluminescent methods. **Results:** Ven was found to have a protective effect against the Ca^{2+}-induced mPTP opening, increasing the threshold calcium concentrations and the calcium load that activate pore opening. A comparison with the known mPTP inhibitors ADP, bongkrekic acid (BA), cyclosporine A (Cs), oligomycin, used separately and in combination with Ven, showed a similarity of the effect of Ven (at a concentration of 50 µM) to the effect of BA (20 µM). The effects of both were prevented by carboxyatractyloside (CATR). Also, the protective effect of Ven, as well as that of BA, was completely eliminated by 2-thenoyltrifluoroacetone (TTFA), an inhibitor of the ubiquinone-binding site of succinate dehydrogenase complex at low concentrations (5 µM and 50 µM under the oxidation of succinate and NAD-dependent substrates, respectively). Simultaneously, Ven decreased the ROS production induced by TTFA under the same conditions. **Conclusions:** Ven shows two protective activities, namely, the inhibition of mPTP opening and the suppression of ROS production in mitochondria, which may be additional causes of the development of drug resistance. The results of the TTFA test are consistent with the data on the dependence of Ven resistance on the state of the respiratory chain.

Keywords: venetoclax; drug resistance; mitochondria; mitochondrial permeability transition pore; bongkrekic acid; reactive oxygen species; cell respiration; succinate dehydrogenase

1. Introduction

Numerous data show that there are direct relationships between mitochondrial activity, cancer progression, and chemoresistance. Mitochondrial dysfunctions found in various types of cancer are mostly associated with tumor progression and development of chemoresistance [1]. Since mitochondria can adapt to pathological conditions to provide cell survival, they are currently considered as potential targets for cancer therapy [2,3]. As shown, many interdependent functions of mitochondria, including redox regulation, calcium signaling, biosynthesis of essential intermediates, and regulation of apoptosis are important for the survival of cancer cells [4]. Mitochondria are involved in survival pathways via the modification of the oxidative stress response, mitochondrial dynamics, mitophagy, and Ca^{2+} homeostasis [3,4].

In addition to the mitochondrial protective mechanisms of cell survival, ATP-binding cassette transporters (ABC transporters) are generally considered as the major causes of drug resistance, providing the ATP-dependent efflux of truly effective medications across plasma membranes. These transporters hydrolyze ATP, bind drugs, and promote their translocation across a plasma membrane [5,6,7]. The inhibition of transporters enhances the intracellular accumulation and retention of drugs. There is evidence indicating the dependence of the effect of some inhibitors of multidrug resistance (MDR)-related proteins on mitochondrial processes [8,9]. We have shown that the action of quinidine and verapamil, the first-generation MDR inhibitors, extends to mitochondria, in particular, to the nonspecific membrane permeability [10]. Besides, tariquidar, a more hydrophobic compound from the latest-generation inhibitors, which is characterized by a high

Copyright: © 2026 The Author(s). Published by IMR Press.
This is an open access article under the CC BY 4.0 license.

Publisher's Note: IMR Press stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

affinity for ABC proteins, decreased the capacity of mitochondria to accumulate calcium ions, thus supporting apoptosis [11].

Venetoclax (Ven) is a mitochondria-targeted chemotherapeutic agent, which facilitates apoptosis by selectively inhibiting the antiapoptotic protein Bcl-2. Ven specifically mimics and replaces the BH3 domain (Bcl-2 homology domain 3) of Bcl-2 [12]. This effect limits the ability of Bcl-2 to bind the proapoptotic proteins BAX and BAK, which provide the mitochondrial outer membrane permeability, promoting the mitochondrial apoptotic pathway [13]. It was also shown that, regardless of this selective Bcl-2 inhibition, Ven can influence the cellular metabolism and thereby modulate the cytotoxicity of the drug [14]. In addition, Ven shows a dual effect, affecting not only mitochondria but also the cell drug resistance system. It partially reverses multidrug resistance and increases the intracellular accumulation of chemotherapeutic drugs, directly inhibiting the ATPase activity and blocking the efflux of the drug due to its high binding affinity for the transporter [15,16,17]. At the same time, there is also evidence indicating that cancer cells develop resistance to Ven. It has been revealed that the resistance to Ven can be mediated by the upregulation of antiapoptotic proteins, simultaneous activation of multiple signaling pathways, altered cellular metabolism, mitochondrial metabolic reprogramming, and activation of oxidative phosphorylation [18]. In particular, it was noted that Ven-sensitive multiple myeloma, in contrast to Ven-resistant one, exhibits reduced cellular energetics [19].

The influence of Ven on the opening of the mitochondrial permeability transition pore (mPTP) has not been previously studied, although mPTP opening is a critical event in the mitochondria-mediated apoptosis. We have examined the effect of Ven on calcium-induced mPTP opening in healthy isolated rat liver mitochondria and have found that Ven shows a protective effect, increasing the threshold calcium concentrations and calcium load that activate pore opening. The structure of the mPTP complex remains unknown, but several proteins have been proposed to form the pore. Among them, ATP synthase and adenine nucleotide translocase (ANT) are the most probable pore-forming components, while the matrix peptidyl-prolyl cis–trans isomerase cyclophilin D can regulate the Ca^{2+} sensitivity of the components and mPTP opening [20,21]. We used their regulators and inhibitors to identify the target of the action of Ven on the mPTP. In addition, given the known dependence of the action of Ven on the state of the respiratory chain, we examined its effect in the conditions of a partial inhibition of the ubiquinone-binding site by low concentrations of 2-thenoyltrifluoroacetone (TTFA), an inhibitor of the succinate dehydrogenase complex.

2. Materials and Methods

2.1 Materials

Venetoclax was obtained from TOCRIS (Tocris Biosciences, Bristol, United Kingdom). All other reagents (carboxyatractyloside, bongkrekic acid, oligomycin, cyclosporine A, and others) were from the Sigma–Aldrich Corporation (St. Louis, MO, USA) (**Supplementary Table 1**).

2.2 Animals

The study was conducted in accordance with the ethical principles formulated in the Federal Law of the Russian Federation No. 498-FZ 'On Responsible Treatment of Animals'. Manipulations were carried out in agreement with Federal Standard 'Guidelines for the maintenance and care of laboratory animals' (GOST 33215-2014, GOST 33216-2014, and SP 2.2.1.3218-14) by the certified staff of the Animal Department at the Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences. Rats were killed by guillotining after CO_2 anesthesia following Protocol No. 01/2026, approved 25 February 2026 by the Commission on Biomedical Ethics of ITEB RAS.

Experiments were performed on healthy male Wistar rats (12 ± 3 weeks old, weighing 220 ± 10 g), (n = 30). The study design did not include randomization and blinding. The animals were housed under standard laboratory conditions at a temperature of 22 ± 2 °C, relative humidity of 40–70%, and a 12-hour light/dark cycle, with free access to water and a standard pelleted extruded diet (Vivarium-lab, Russia). The room was ventilated, and bedding was changed every 4 days. According to the AVMA 2020 guidelines, anesthesia was induced using carbon dioxide (CO_2) at an air displacement rate of 50% of the chamber volume per minute. The CO_2 exposure time was 3 minutes, after which, the animals were removed from the chamber, guillotinated, and taken for liver excision. The assays were performed on freshly isolated mitochondria immediately after their isolation.

2.3 Isolation of Rat Liver Mitochondria

The liver tissue was immediately minced and homogenized in an ice-cold buffer containing 300 mM sucrose, 10 mM Tris-HCl, and 1 mM EGTA, pH 7.4; all subsequent manipulations were also carried out at 0–4 °C to minimize enzyme activity. The resulting homogenate was centrifuged at 600 ×g for 7 min to pellet nuclei, unbroken cells, and large cell debris, after which the supernatant was carefully collected for further centrifugation. The collected supernatant was centrifuged at 9000 ×g for 10 min to pellet the intact mitochondrial fraction, and the supernatant containing cytosolic components and microsomes was discarded. The resulting mitochondrial pellet was washed twice with the above buffer without EGTA (300 mM sucrose, 10 mM Tris-HCl, pH 7.4), with repeated centrifugation at 9000 ×g for 10 min after each wash. The final mitochondrial pellet

was resuspended in a small volume of the same EGTA-free medium to achieve a suspension concentration of 60 mg protein per 1 mL (Lowry test), after which the mitochondrial suspension was kept on ice and used within 3–4 hours to maintain maximal functional activity.

2.4 Measurement of the Membrane Potential and the Calcium Retention Capacity of Mitochondria

The voltage difference across the membrane was determined by the distribution of the lipophilic cation tetraphenylphosphonium (TPP+), whose concentration in the medium was recorded with a TPP+-selective electrode. The accumulation of Ca^{2+} in the mitochondria was recorded with a Ca^{2+}-selective electrode as a change in the calcium concentration in the external medium in response to successive $CaCl_2$ additions with a Record 4 device (Russia). The calcium retention capacity (CRC) of mitochondria was determined by the ability of mitochondria to accumulate successive additions of calcium ions to the threshold concentration necessary for the opening of the nonspecific mitochondrial pore. The standard incubation medium contained 125 mM KCl, 1.5 mM KH_2PO_4, and 15 mM HEPES (pH 7.25); the mitochondrial protein concentration was 1 mg/mL. Other experimental conditions are indicated in the captions to the figures. The figures show the data of typical experiments performed in at least five replicates with different samples of mitochondria.

2.5 Determination of Mitochondrial Respiration

Oxygen consumption in a mitochondrial suspension was determined by the polarographic method with a Clark-type electrode in a closed chamber of 2 mL containing 2.0 mg of mitochondrial protein under continuous stirring. Mitochondrial respiration was supported by succinate (5 mM) and glutamate plus malate (5 mM). Respiration was activated by ADP (200 – 250 μM) for the evaluation of coupled respiration and by 500 nM FCCP for the evaluation of uncoupled respiration. The respiratory control was defined as the ratio of the ADP-stimulated respiration rate to the respiration rate after ADP phosphorylation.

2.6 Assay for Mitochondrial V3 Respiration-Like Activity

In order to detect weak effects of Ven on the mitochondrial oxidative phosphorylation system, we applied a potassium ferricyanide $K_3Fe(CN)_6$ (FCN) model, which involves the assessment of the rate of the substrate-dependent FCN reduction. This approach allows one to record changes in the rate of electron transfer in segments of complexes I and III and complexes II and III of the mitochondrial respiratory chain upon the addition of ADP. Being a membrane-impermeable acceptor, FCN takes electrons directly from cytochrome c, which decreases the overall efficiency of proton pumping ($H^+/2e^-$ ratio) by the respiratory chain to ~6 (complex I and III segment) and ~2 (complex II and III segment) and thus makes visible small changes in the efficiency of ADP phosphorylation. The reduction of FCN was detected by a decrease in absorption at 405 nm using the plate readers Infinite 200 Tecan and Infinite 200 Tecan Pro (Groedig, Austria) and 96-well plates. KCl-based incubation medium contained 2 mM $MgCl_2$, 2 mM KH_2PO_4, 1 mM EGTA, 1 mM KCN, and 2 mM $K_3Fe(CN)_6$. Other details are given in the figures and figure legends.

2.7 Assessment of ROS Production

The production of ROS by mitochondria was monitored by optical methods in suspension using the plate readers Infinite 200 Tecan or Infinite 200 Tecan Pro (Groedig, Austria) and 96-well plates as described earlier [22].

2.7.1 H_2O_2 Release

Resorufin accumulation (which linearly depends on H_2O_2 production) was traced at excitation and emission wavelengths of 530 and 595 nm. For the quantitative measurement of H_2O_2, fluorescence was calibrated by the addition of an excess of H_2O_2 (100 μM final concentration) to several wells at the beginning and at the end of the recording. This was done to assess the rate of the conversion of Amplex Red (AR)/resorufin to a nonfluorescent product [23] and thus to deduce the fluorescence of 40 μM resorufin at zero time.

2.7.2 Superoxide Anion Release

The production of $O_2^{\cdot-}$ was monitored using the ΔΨm-insensitive and highly selective probe 6-(4-methoxyphenyl)-2-methylimidazo[1,2-a]pyrazin-3(7H)-one hydrochloride (MCLA) [24,25,26]. MCLA-derived chemiluminescence (MDCL) was recorded approximately once per minute. Each value on the curve is the mean ± SD of three integrations of luminescence for 900 ms expressed in arbitrary units (AU). In order to separate relatively bright spontaneous $O_2^{\cdot-}$ insensitive MDCL from $O_2^{\cdot-}$ sensitive one, some wells contained SOD at indicated concentrations. Since MDCL in solution is effectively quenched by compounds that contain divalent sulfur [27], in preliminary studies we evaluated the effect of Ven (which contains hexavalent sulfur) on the spontaneous MDCL and MDCL in a xanthine/xanthine oxidase system. Though this effect was very limited, we took it into account in the data analysis. Since MDCL reflects the quantity of photons emitted by the excited oxy-MCLA within a 900-ms period of luminescence accumulation at each experimental point (AU accumulated per 900 ms), MDCL is the measure of the rate of $O_2^{\cdot-}$ production. As the rate of $O_2^{\cdot-}$ production by mitochondria is not constant during long incubation, in order to assess the net $O_2^{\cdot-}$ production over a long period, we integrated MDCL values within the initial 10–60-min interval of incubation. Other details are given in the figures and figure legends.

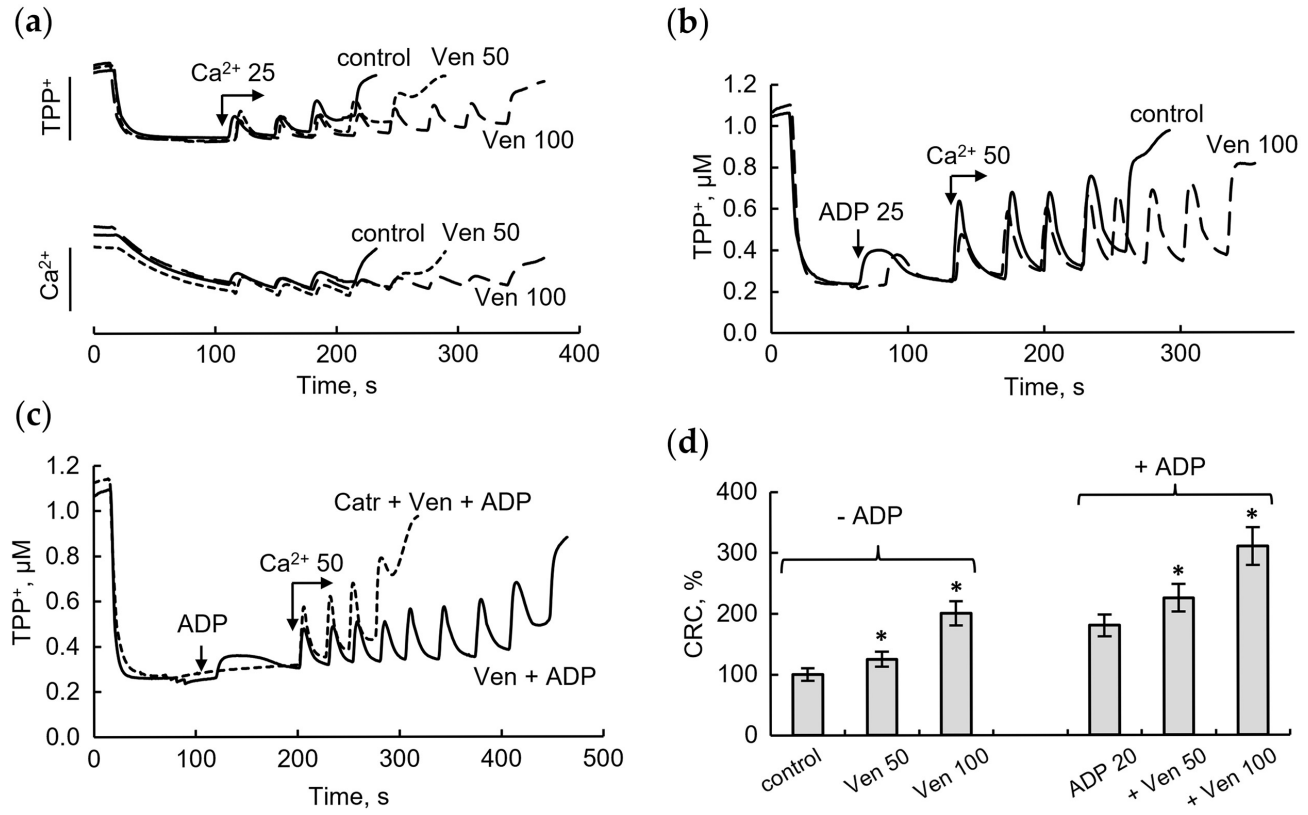

Fig. 1. Effect of venetoclax on the calcium accumulation by mitochondria in the control and in the presence of ADP. The influence of venetoclax (Ven) on the calcium retention capacity (CRC), assessed by a drop in the membrane potential (tetraphenylphosphonium [TPP] scale) and calcium release (Ca scale) during successive additions of $CaCl_2$ in the control (a), in the presence of 25 µM ADP (b) and 5 µM Carboxyatractyloside (CATR, c). Changes in the CRC depending on the concentration of Ven in the control and with 20 µM ADP (d). Asterisks indicate the values that differ significantly from the control values ($p < 0.05$).

2.8 Statistical Analysis

The measurements of CRC and membrane potential were performed in three series (Ven with ANT inhibitors, combinations of mPTP modulators, and TTFA plus mPTP modulators including Ven) with 5–7 independent experiments (5–7 mitochondrial preparations) for each series. Measurement of ROS production and V3 Respiration-Like Activity included 3–5 separate experiments and three technical replicates for each experimental condition. All data are presented as means ± standard deviation (SD) from 3–7 independent experiments, unless otherwise stated. Representative traces are shown from at least three independent experiments, as specified in the corresponding figure legends. Arithmetic means of technical replicates were taken for determination of statistical significance using the paired Student's t test, with $p < 0.05$ considered significant. All statistical analyses and graphical presentations were performed using Microsoft Excel (version 16.0, Microsoft Corp., Redmond, WA, USA).

3. Results

First, we examined whether Ven is capable of influencing calcium-induced mPTP opening, which was assessed by the ability of mitochondria to accumulate calcium during its successive additions. Ven was found to exert a protective effect by increasing the threshold calcium concentrations that induce pore opening, which is evident from a drop in the membrane potential and the release of calcium from mitochondria (Fig. 1a). The effect increased with increasing Ven concentration, and, at a concentration of 100 µM, the CRC increased almost twofold compared with the control. Ven also enhanced the effect of ADP, an inhibitor of mPTP opening. In the presence of ADP, Ven increased the mitochondrial resistance to calcium loading by approximately 50% (Fig. 1b,d). These effects were eliminated by the ANT inhibitor carboxyatractyloside (CATR) (Fig. 1c). Thus, Ven itself acts as a protector against the opening of the mitochondrial pore and enhances the protective effect of ADP (Fig. 1d).

To localize the probable targets of the action of Ven on the mPTP complex, we examined the effect of mPTP inhibitors, individually and in combination with Ven. Of particular interest was bongkrekic acid (BA), an ANT and mPTP inhibitor, which acts similarly to ADP. As expected, BA at a standard concentration of 20 µM inhibited pore opening, doubling the CRC (Fig. 2a). In combination with

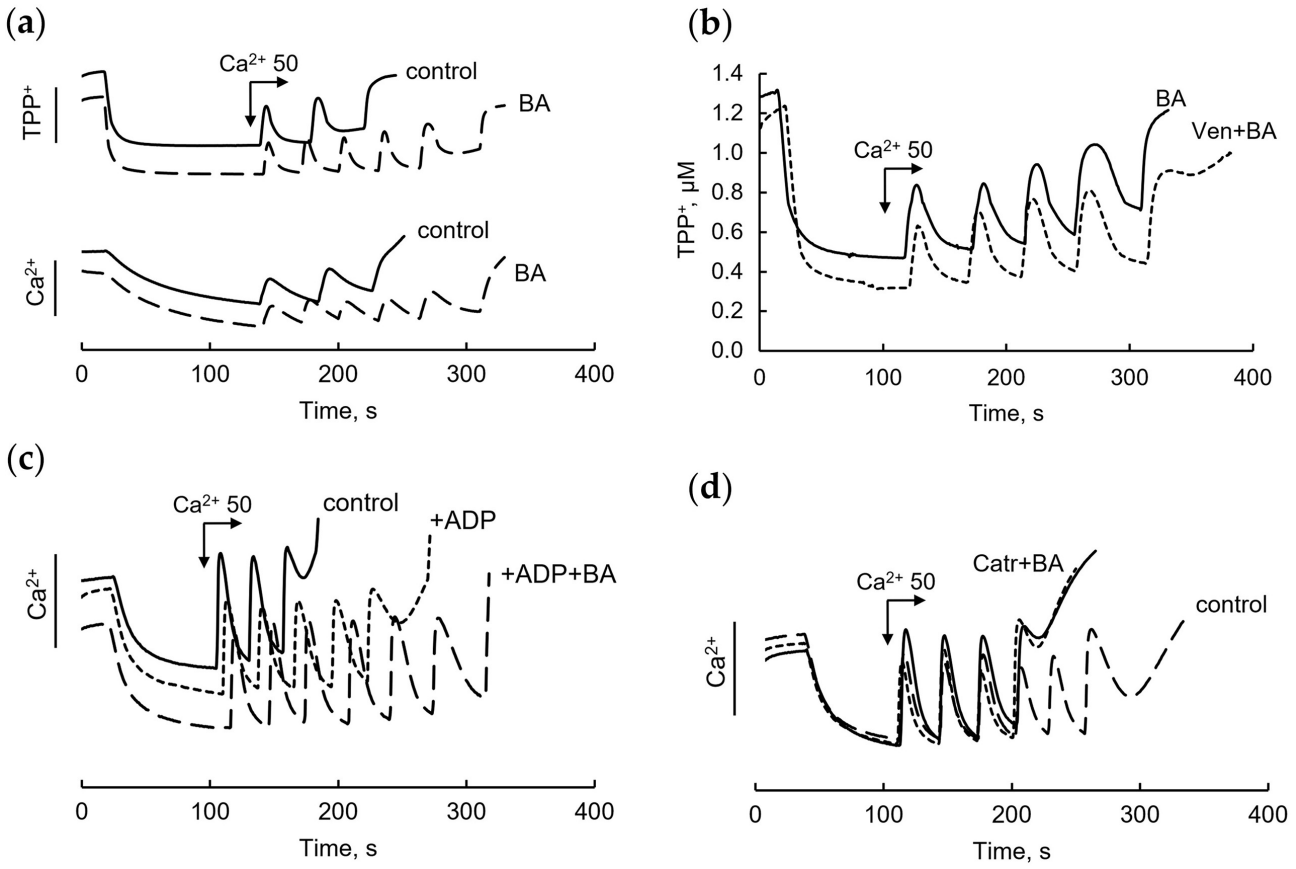

Fig. 2. Influence of venetoclax in combination with bongkrekic acid on mPTP opening. The calcium retention capacity (CRC), assessed by a drop in the membrane potential (TPP scale) or calcium release (Ca scale) during successive additions of $CaCl_2$ (50 μM). Effect of BA (20 μM) alone on the CRC in the control (a), with 50 μM venetoclax (b), with 25 μM ADP (c), and 5 μM CATR (d). The typical traces of five identical experiments with the use of different mitochondrial samples are presented. mPTP, mitochondrial permeability transition pore; BA, bongkrekic acid.

Ven, the effect of BA on mPTP did not change (Fig. 2b). However, BA significantly enhanced the protective effect of ADP on mPTP opening (Fig. 2c). The ANT inhibitor CATR removed the protective effect of BA (Fig. 2d).

The inhibitors of other components of the mPTP complex, namely, oligomycin as an ATPase inhibitor, and cyclosporine A (Cs) as an inhibitor of cyclophilin D, were also tested, individually and in combination with Ven. As shown in Fig. 3a, oligomycin did not change the sensitivity to calcium load, since the CRC remained the same as in the control. However, oligomycin enhanced the protective effect of ADP (Fig. 3a). Also, oligomycin increased the CRC in the presence of both Ven and BA (Fig. 3b). Thus, without affecting the mPTP by itself, oligomycin promoted the protective effect of BA, Ven, and ADP.

The results of experiments with the combined action of cyclosporine with Ven and other mPTP inhibitors turned out to be unexpected. Although Cs is the most potent inhibitor of mPTP, all inhibitors further increased the CRC in its presence. As shown in Fig. 3c, Ven showed only a slight effect in this regard. ADP had a stronger effect, increasing the resistance to calcium load by more than 50%. But BA exerted the strongest influence. As shown in Fig. 3d, BA increased calcium resistance twofold compared to Cs alone.

These experimental data show the protective effect of Ven against the Ca-induced mPTP opening. Fig. 4 summarizes the data on the effect of both Ven itself and in combination with other known mPTP inhibitors.

In the following experiments, we examined the effect of Ven on mitochondrial respiration and oxidative phosphorylation. As shown in Fig. 5, Ven only weakly affected the respiration rate during the oxidation of succinate and had no effect on the respiration upon oxidation of the NAD-dependent substrates glutamate and malate. The respiratory control decreased slightly upon the oxidation of succinate.

Therefore, we assessed the effect of Ven in a ferricyanide (FCN) model, where the membrane-impermeable acceptor received electrons directly from cytochrome c, decreasing the overall efficiency of proton pumping (the $H^+/2\bar{e}$ratio) by the respiratory chain (Fig. 6). Fig. 6a,b show the control curves of the reduction of the acceptor in response to ADP addition in the presence of succinate (Fig.

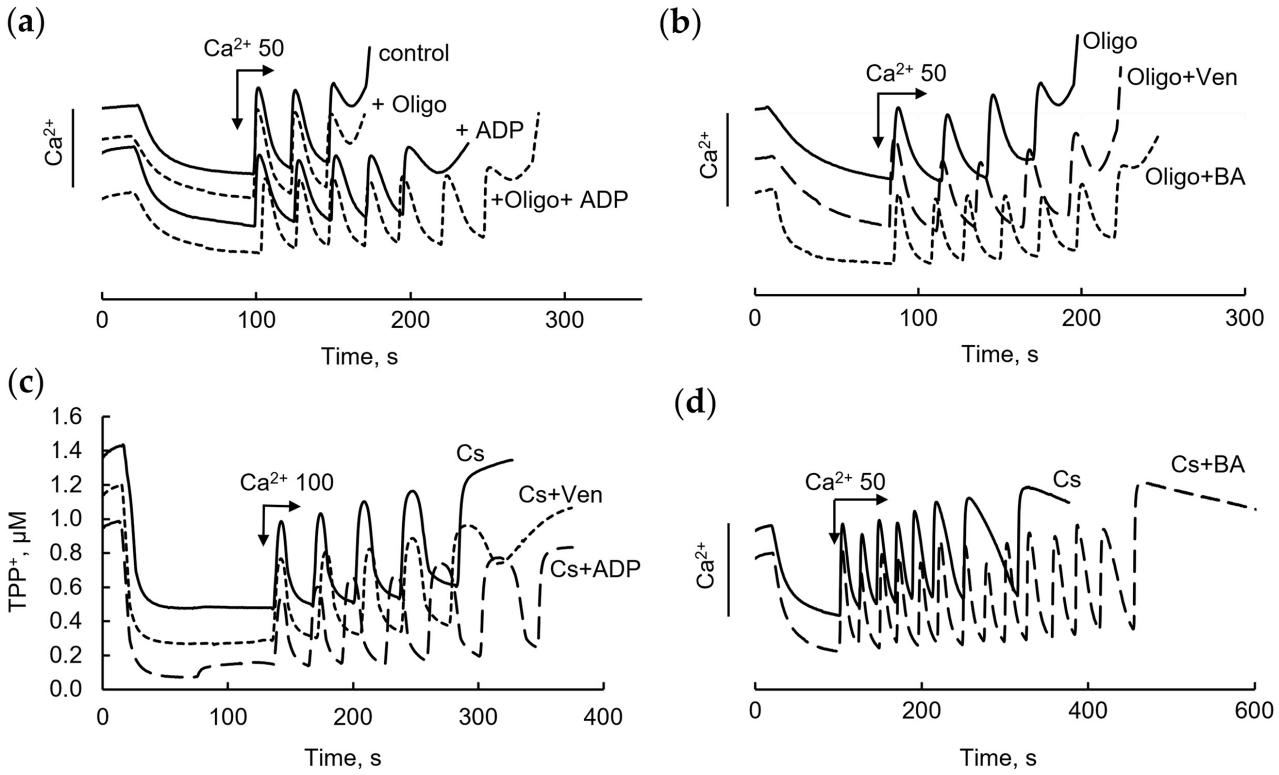

Fig. 3. Influence of venetoclax and bongkrekic acid on mPTP opening in the presence of oligomycin and cyclosporine. Calcium retention capacity (CRC) assessed by the drop in membrane potential (TPP scale) or calcium release (Ca scale) during successive additions of $CaCl_2$ (50 μM and 100 μM, as indicated). The influence of 5 μM oligomycin on CRC in control and in the presence of 50 μM ADP (a); of 50 μM Ven and 20 μM BA on CRC in the presence of oligomycin (b); the influence of Ven (50 μM) and ADP (50 μM) (c) and BA (20 μM) (d) on mPTP opening in the presence of Cs (2 μM). The typical traces of three to five identical experiments with the use of different mitochondrial samples are presented.

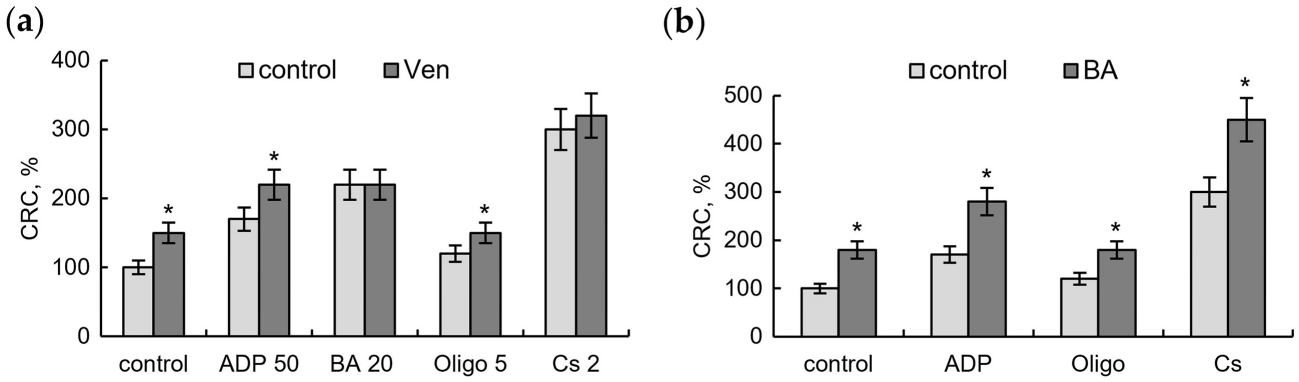

Fig. 4. Influence of venetoclax and bongkrekic acid in combination with different mPTP inhibitors on the resistance to calcium load. Calcium retention capacity (CRC) in the presence of the indicated inhibitors alone (light columns) and in combination (dark columns) with 50 μM Ven (a) and 20 μM BA (b). The concentrations of reagents are given in μM. Values on columns are means ± SD (n = 5). Asterisks indicate the values that differ significantly compared with the corresponding values of inhibitor-alone condition ($p < 0.05$).

6a) and glutamate plus malate (Fig. 6b). ADP at high concentration (500 μM) stimulated oxidative phosphorylation and, accordingly, the reduction of FCN. The FCN reduction was more prominent with succinate than with NAD-dependent substrates. This could be expected, since the number of protons pumped out per pair of electrons is approximately three times lower in the presence of succinate, and, hence, proportionally more FCN must be reduced to

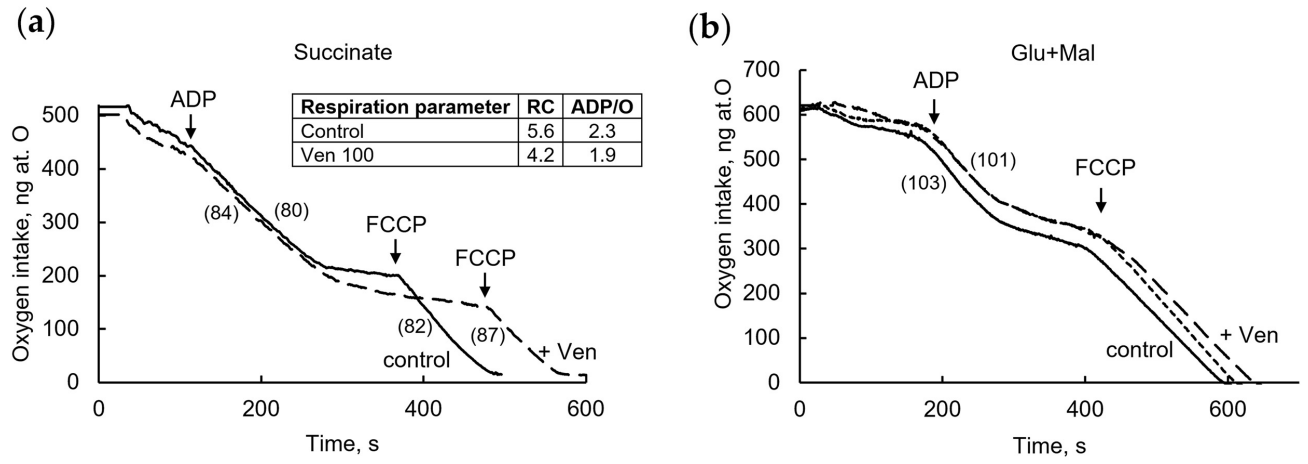

Fig. 5. Influence of venetoclax on the respiration rates and oxidative phosphorylation. Effect of Ven (100 μM) on oxygen consumption during the oxidation of succinate (a) and glutamate plus malate (b). Where indicated, ADP (250 μM) and 500 nM carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) were added. The inset shows changes in respiratory control (V_3/V_4) and ADP/O in the presence of Ven during succinate oxidation. Respiration rates are indicated in parentheses; the typical traces of five identical experiments with the use of different mitochondrial samples are presented.

create the H^+ gradient sufficient for ADP phosphorylation (a lower ADP/FCN ratio). As shown in Fig. 6c,d, the ANT inhibitors CATR and BA suppressed the ADP-stimulated electron transfer, decreasing the rate and the amplitude of FCN reduction during the oxidation of substrates of both respiratory chain complexes. Ven acted in the same direction, but the effect was observed only during succinate oxidation.

Note that, in permeable mitochondria, FCN takes electrons either from SDH, bypassing ubiquinone, or from the iron-sulfur (Fe-S) clusters of complex I. Therefore, mPTP opening in a portion of mitochondria will stimulate FCN reduction and inhibit the respiration.

Thus, these data indicate that Ven produces the most pronounced effect upon the oxidation of succinate, a substrate of the respiratory chain complex II. Next, we analyzed the role of respiratory chain dysfunction in the influence of Ven on mPTP opening. For this purpose, we used TTFA, an inhibitor of the ubiquinone-binding site of the SDH complex.

Fig. 7 shows the effect of TTFA on calcium-induced mPTP opening. It is seen that TTFA decreased the CRC in a concentration-dependent manner. The TTFA concentration that decreased the threshold sensitivity to calcium varied depending on the oxidation substrate. Thus, while at a concentration of 10 μM, TTFA decreased the CRC by about fourfold during the oxidation of succinate (Fig. 7a), a similar effect during the oxidation of NAD-dependent substrates was achieved only at a concentration of TTFA of 50 μM (Fig. 7b). Interestingly, even at a lower TTFA concentration (2.5 μM), which reduced the calcium threshold only about twofold, none of the mPTP inhibitors (Ven, BA, and ADP) increased the CRC (Fig. 7c,d). Under these conditions, only Cs retained its effect as an inhibitor of mPTP opening (Fig. 7e).

Since TTFA, as an inhibitor of the respiratory chain in the ubiquinone-binding site, can provoke oxidative stress, we tested the possible influence of Ven on the TTFA-induced ROS production. This is a second aspect of the possible involvement of Ven in mPTP regulation because oxidative stress promotes pore opening. Ven alone slightly stimulated superoxide anion release in the presence of succinate but had no statistically significant effect in the presence of glutamate plus malate. TTFA activated the superoxide production (20 μM menadione is given as a standard for superoxide formation) upon the oxidation of both succinate (Fig. 8a) and glutamate plus malate (Fig. 8b). As in the experiments on calcium-induced mPTP opening, the effective TTFA concentrations for substrates of complexes I and II differed tenfold: 50 μM for glutamate plus malate and 5 μM for succinate. It turned out that Ven diminished the superoxide production in the presence of both succinate and glutamate plus malate, bringing it closer to control values. SOD, used as a control, completely blocked the MDCL reduction. Comparable indicators of ROS production under the specified conditions are shown in Fig. 8c and d; the data are presented for the oxidation of succinate (Fig. 8c) and glutamate with malate (Fig. 8d).

A similar result was observed when testing the effect of Ven on hydrogen peroxide production. It is noteworthy that, despite the activation of superoxide production, TTFA did not activate H_2O_2 production (Fig. 8e). However, a spontaneous increase in the concentration of H_2O_2 occurred during relatively prolonged incubation of mitochondria, which was confirmed by the inhibition of AR oxidation during incubation with catalase. Ven acted in the same

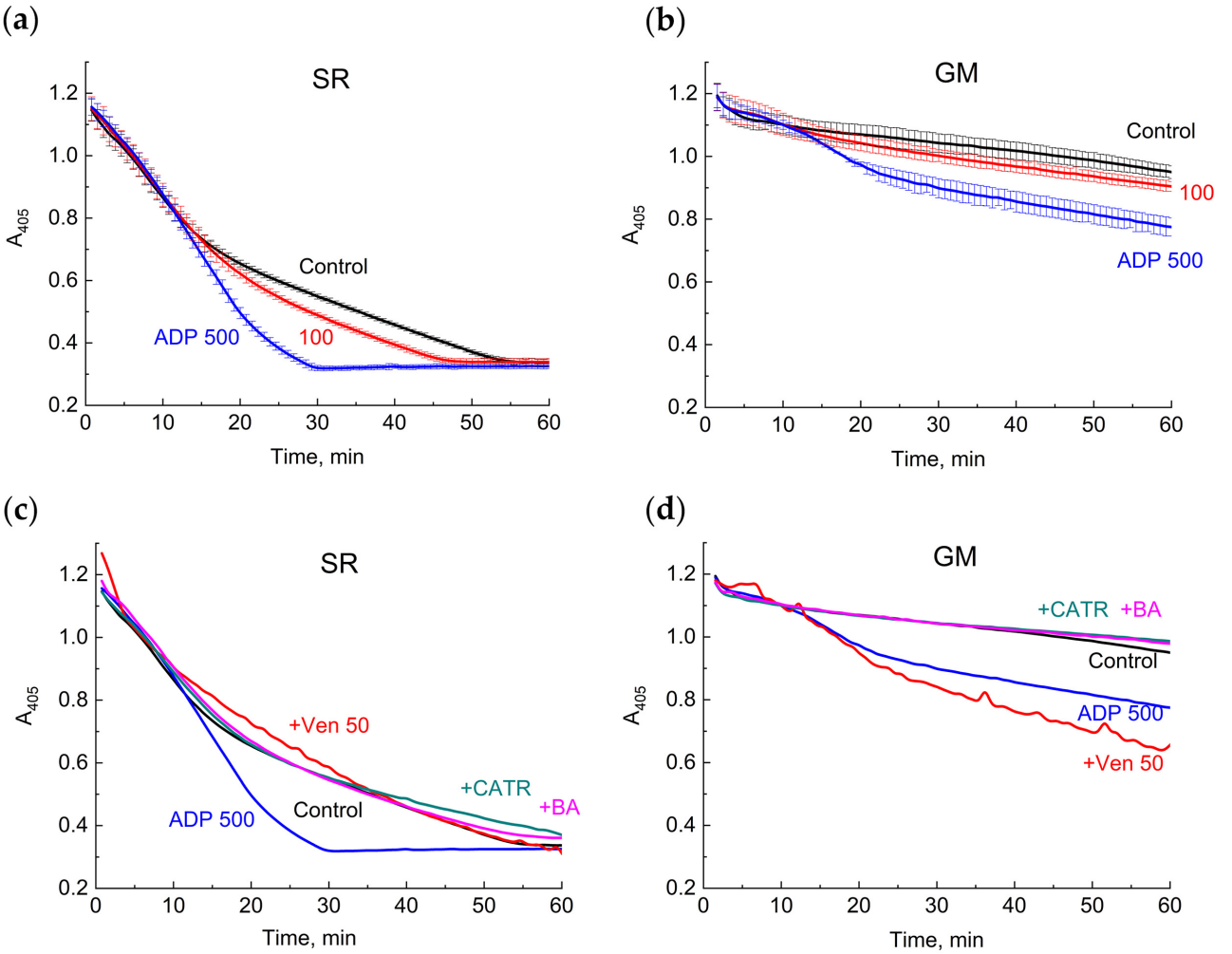

Fig. 6. Effect of ADP, Ven, and ANT inhibitors on the rate of FCN reduction. RLM (0.2 mg protein/mL) were placed in KCl-based medium supplemented with 2 mM $MgCl_2$, 2 mM KH_2PO_4, 1 mM EGTA, 1 mM KCN, 2 mM $K_3Fe(CN)_6$, and, where shown, 5 mM succinate plus rotenone (2 µg/mL) (SR) (a,c) or 5 mM glutamate plus 5 mM malate (GM) (b,d). After 1-min incubation, suspension was transferred to the wells of a plate, which contained, where indicated, Ven (50 µM), ADP (100 and 500 µM), 1 µM CATR, and 10 µM BA. (a–d) Standard traces of one representative experiment of three similar. Numbers at the traces show the concentrations in µM. Points on the curves are the means ± SD of three technical replicates. ANT, adenine nucleotide translocase; FCN, ferricyanide; RLM, rat liver mitochondria.

direction as catalase, weakly decreasing AR oxidation regardless of the presence of TTFA (Fig. 8e,f). Since MDCL in solution is effectively quenched by compounds having divalent sulfur, we tested a possible role of Ven, which contains hexavalent sulfur, in these experiments. Fig. 8g shows that the influence of Ven on the spontaneous MDCL and MDCL in a xanthine/xanthine oxidase system is small but measurable. Therefore, the data in panels a–d were corrected accordingly.

4. Discussion

Venetoclax is a mitochondria-targeted chemotherapeutic drug, which facilitates apoptosis through the selective inhibition of the antiapoptotic protein Bcl-2. It is widely used for the treatment of myeloid leukemia, as well as hematologic and other tumors in which the protein Bcl-2 is expressed at a high level [13,28,29]. Bcl-2 promotes cell survival, blocking the mitochondrial outer membrane permeabilization (MOMP) via binding the proapoptotic proteins of the BAX/BAK family. The proteins BAX/BAK oligomerize and permeabilize the outer mitochondrial membrane, releasing cytochrome c to activate the subsequent steps of apoptosis [30,31]. In addition, the Bcl-2 family is considered a new class of intracellular calcium (Ca^{2+}) regulators, since they are able to interact with major Ca^{2+} transporters, thus controlling the mitochondrial Ca^{2+} homeostasis and downstream Ca^{2+} signaling pathways [32]. Moreover, it has been shown that resistance to

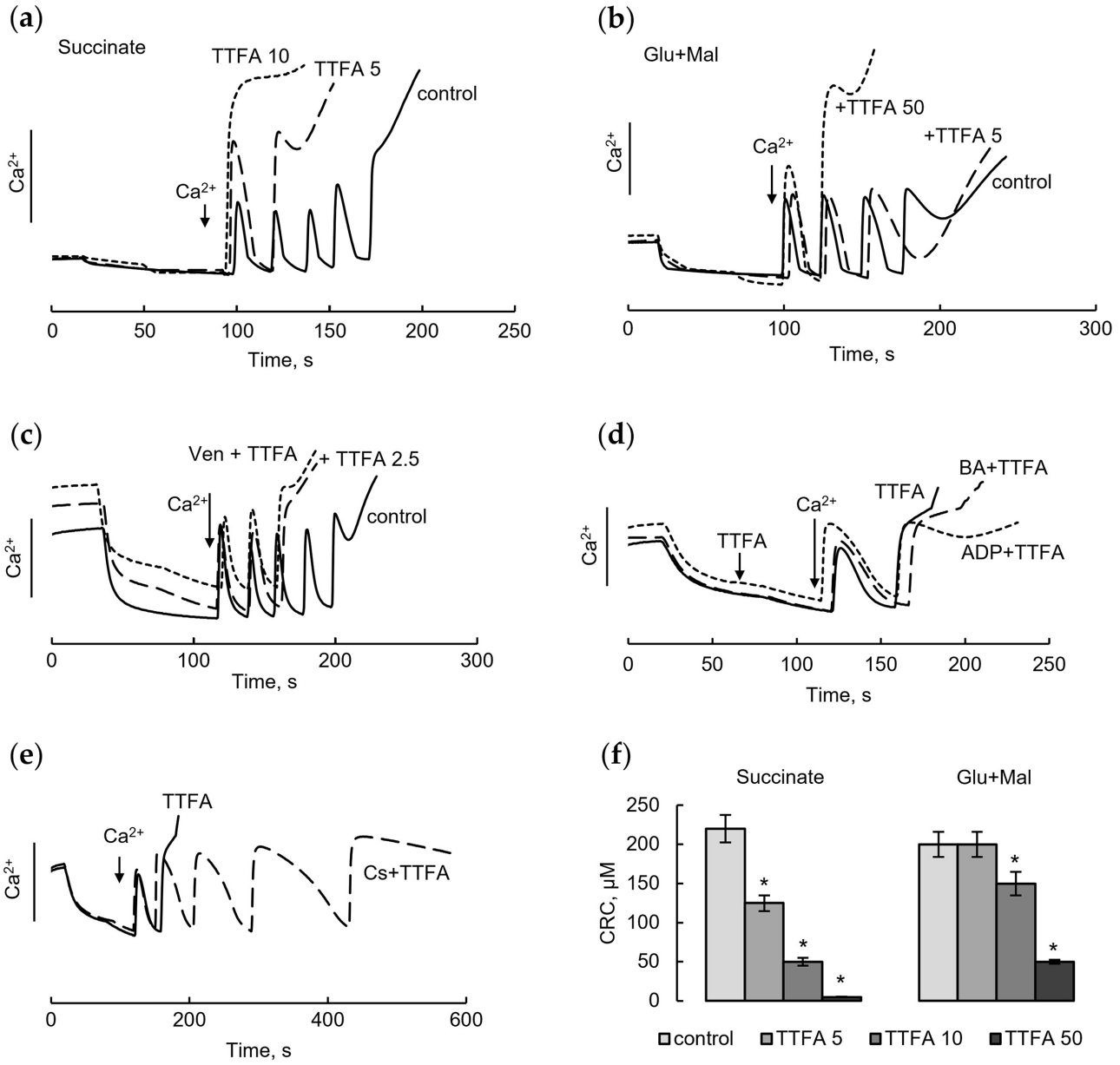

Fig. 7. Effect of Ven and mPTP inhibitors on calcium-induced mPTP opening in the presence of the respiratory chain inhibitor TTFA. The effect of TTFA at the indicated concentrations alone on calcium capacity during successive additions of calcium (25 μM) upon the oxidation of succinate (a,c–f) and glutamate plus malate (b,f) and in the presence of 100 μM Ven (c), 20 μM BA and 25 μM ADP (d) and 2 μM Cs (e). The concentrations of TTFA at the traces are given in μM. In panels (c–e) it is equal to 2.5 μM. Differences in TTFA concentrations in the effect on the CRC upon the oxidation of succinate and NAD-dependent substrates (f). The typical traces of three to five identical experiments with the use of different mitochondrial samples are presented. Values on columns are means ± S.D. (n = 5). Asterisks indicate the values that differ significantly from the control values ($p < 0.05$). TTFA, 2-thenoyltrifluoroacetone.

Ven is associated with intrinsic differences in calcium signaling pathways, including differences in the steady-state levels of mitochondrial calcium, which were significantly higher in Ven-resistant myeloid leukemia stem cells [33]. Interestingly, mitochondria largely determine the sensitivity of cancer cells to Ven. In resistant myeloma cells, mitochondrial respiration, oxidative phosphorylation, and the membrane potential are maintained at normal physiological levels, while Ven-sensitive cells show suppressed mitochondrial bioenergetics [34]. According to some data, Ven has an inhibitory influence on the respiratory chain and the tricarboxylic acid cycle in the mitochondria of multiple myeloma cells [14,19,34].

Our experiments revealed a new property of Ven associated with the regulation of mPTP opening by calcium ions. Ven exerted a protective influence on the pore open-

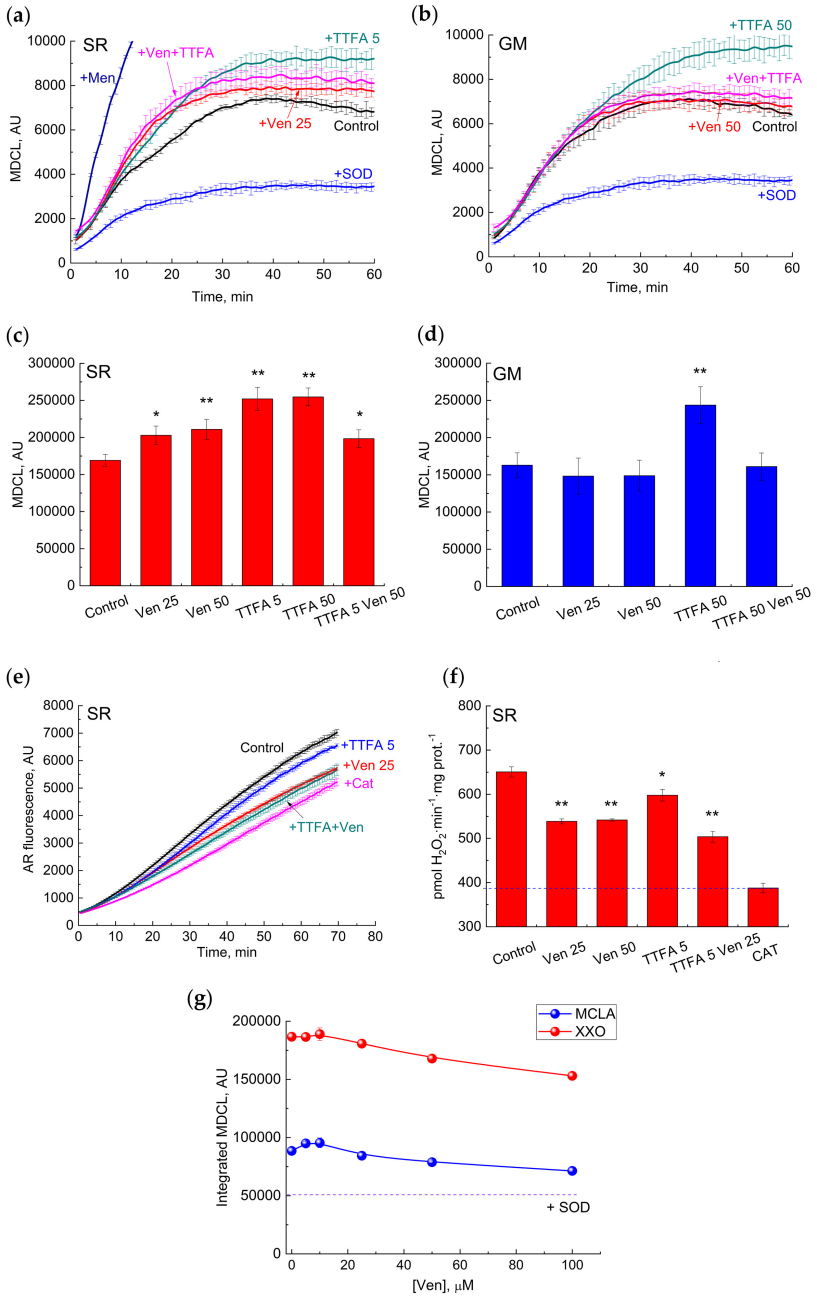

Fig. 8. Effect of Ven and TTFA on the production of superoxide anion (a–d) and H_2O_2 (e,f) by mitochondria. (a–d) RLM (0.75 mg protein/mL) were placed in KCl-based medium supplemented with 2 mM $MgCl_2$, 2 mM KH_2PO_4, 1 mM EGTA, 20 μM MCLA, and, where shown, 5 mM succinate plus rotenone (2 μg/mL) (SR) or 5 mM glutamate plus 5 mM malate (GM). After 1-min incubation, the suspension was transferred to the wells of a plate, which contained, where indicated, TTFA (5 or 50 μM), Ven (25 or 50 μM), 20 μM menadione (Men) and SOD (200 U/mL). (a,b) Standard traces of one representative experiment of three similar. Points on the curves are the means ± SD of three technical replicates. (c,d) Integrated SOD-sensitive part of MDCL (10–60 min) (mean ± SD, n = 3 (three independent experiments, each with three technical replicates); * - $p < 0.05$; ** - $p < 0.01$). (e,f) RLM (0.5 mg protein/mL) were placed in the medium as in a and c, except that 40 μM AR and HRP (3 U/mL) were added instead of MCLA. After 1-min incubation, the suspension was transferred to the wells of a plate, which contained, where indicated, 5 μM TTFA, Ven (25 and 50 μM), and catalase (Cat, 1000 U/mL). (e) Standard traces of one representative experiment of three similar. Points on the curves are the means ± SD of three technical replicates. (f) The average rate of H_2O_2 production per 40-min measurement (mean ± SD, n = 3 (three independent experiments, each with three technical replicates); * $p < 0.05$; ** $p < 0.01$). (g) The influence of Ven on the spontaneous MCLA alone and MDCL in the presence of xanthine/xanthine oxidase (XXO) in a mitochondria-free solution. MCLA, 6-(4-methoxyphenyl)-2-methylimidazo[1,2-a]pyrazin-3(7H)-one hydrochloride; MDCL, MCLA-derived chemiluminescence; AR, Amplex Red; SOD, superoxide dismutase.

ing, increasing the resistance to calcium load. This effect of Ven is rather selective, since its influence on other mitochondrial parameters was weaker or absent. As our data showed, Ven did not affect the membrane potential and the respiration rate and only slightly decreased the ADP/O ratio. Moreover, Ven showed another protective effect related to a decrease in superoxide production by mitochondria at certain conditions (TTFA). In our experiments, the effect of Ven was tested on normal isolated mitochondria, which allowed us to evaluate the properties of the drug on its own, rather than those associated with mitochondrial modifications or adaptation to pathology. This approach is well confirmed by our finding that even a slight alteration of the mitochondrial respiratory chain removes the protective effect of Ven on mPTP opening.

It is known that the opening of the mPTP is a critical event in the induction of mitochondria-dependent cell death. Calcium ions are the most potent inducers of the mPTP; therefore, Ca^{2+} buffering in mitochondria is important for the maintenance of mitochondrial integrity, energy production, redox balance, and apoptosis [20]. As our data showed, Ven almost twofold increased the threshold calcium concentrations required to open the pore. A comparison with other mPTP inhibitors, ADP, BA, oligomycin, and Cs, which act on different components of the mPTP complex, showed that the effect of Ven is similar to the action of BA. While in the presence of ADP and oligomycin, Ven further increased the CRC, no such effect was observed in the presence of BA. Presumably, the protective effects of mPTP inhibitors acting at different sites are additive, while those acting at the same site do not potentiate each other. This assumption is generally supported by our experiments evaluating the combined impact of inhibitors (e.g., BA plus oligomycin or Ven plus oligomycin) on the resistance to calcium load. Besides, as previously suggested, BA may increase the affinity to ADP [35]. BA is known to stabilize ANT in the matrix ("m") conformational state and inhibit mPTP opening. In some cases, drug resistance development is associated with the ANT conformational status. Thus, BA increased the chemosensitivity of nasopharyngeal carcinoma cells to cisplatin [8]. The isoform of the third type (ANT3) is considered as a therapeutic target for overcoming progression and resistance in multiple myeloma [9]. Cell sensitivity to apoptosis was altered by both overexpression and knockdown of ANT, so this dependence may be opposite for different cell types [36,37]. Our assumption that Ven influences mPTP opening via an ANT-dependent pathway similarly to the influence of BA is supported by the abolition of the effects of both compounds by the ANT inhibitor CATR, which acts from the cytosolic side of the membrane. On the other hand, the high lipophilicity of Ven suggests its preferential distribution in the mitochondrial lipid membrane and subsequent influence on membrane proteins, including pore-forming components. We have previously shown that lipophilic drugs such as tariquidar and pioglitazone abolish the protective effect of adenine nucleotides (at low concentrations) by acting on ANT [11,38]. The effect of pioglitazone was connected with the slight ANT-mediated protonophoric action [38]. In addition, Ven and BA inhibit pore opening at approximately comparable concentrations, 50 and 20 μM, respectively. At the same time, significant differences between them were revealed in tests with combined effects, especially in their combination with cyclosporine, in which the dominant effect of cyclosporine on mPTP opening was sharply enhanced in the presence of BA and only weakly in the presence of Ven. If we assume that cyclophilin D regulates the Ca^{2+} sensitivity in pore formation, it can be proposed that BA and, to a lesser extent, Ven and ADP increase its threshold, thereby increasing the resistance to mPTP opening. Presumably, cyclophilin D facilitates the Ca^{2+}-dependent transition of mPTP from the closed to an open state [21]. By contrast, BA and, to a lesser extent, Ven and ADP (at low concentration) stabilize the ANT in the m-conformation, unfavorable for mPTP opening, and thus compete with cyclophilin D, enhancing the effect of Cs on pore closure. It seems that such combinations in themselves are of interest for a more detailed study. However, it can now be noted that in all tests, Ven acted as a protector against the opening of the mPTP.

Since Ven interfered with V_3–V_4 transition, possible targets of Ven may be the coenzyme Q-binding sites, which are known to contribute to the mPTP regulation [39,40]. These regulatory sites are thought to be associated with the complex I of the respiratory chain, which may underlie the influence of Ven on superoxide release in the presence of complex I and II substrates. As our results show, TTFA, a mitochondrial complex II inhibitor, removes the protective effects of ANT-targeted inhibitors, including Ven. These data are in good agreement with the previously demonstrated decrease in the resistance of tumor cells (multiple myeloma and murine colorectal cancer cells) to Ven in the presence of respiratory complex inhibitors, especially the succinate ubiquinone reductase inhibitor TTFA [19]. Moreover, the combination of Ven with electron transport chain inhibitors has been suggested as a way to increase the apoptotic potency of Ven [4]. Also, targeting succinate ubiquinone reductase potentiates the efficacy of anticancer therapy by cisplatin. In this case, nontoxic doses of TTFA synergistically stimulated cell death induced by harmless doses of cisplatin in chemoresistant neuroblastoma cells [41]. Indeed, according to our data, low-dose TTFA not only abolished the protective effect of Ven (and other protectors) but also increased the sensitivity of the mPTP to calcium ions. Interestingly, only Cs retained the ability to inhibit mPTP opening under calcium loading in the presence of TTFA. As has been shown, in neuroblastoma cells, the combined effect of TTFA with cisplatin was related to the induction of oxidative stress, since ROS production is one of the signals that sensitize cells to apoptosis [41]. In our

experiments, TTFA induced superoxide production during the oxidation of both succinate and NAD-dependent substrates. However, Ven diminished TTFA-induced increase in superoxide formation, though to a lesser extent than superoxide dismutase did. Ven also showed a similar protective effect on hydrogen peroxide production. These data indicate that Ven may have an antioxidant-like action, probably, associated with the sulfonamide group in its chemical structure. This group demonstrated antioxidant activity in several synthetic sulfonamides [42].

5. Limitations

The protective effect of Ven against the opening of the mPTP represents its new property, which may be significant in some cases of resistance. This effect of Ven can be revealed predominantly in mitochondria with normal respiratory chain activity and cannot extend to transformed mitochondria with modified metabolism, which are often found in different types of cancer cells. It is important to note that effective concentrations of Ven vary significantly between experiments on isolated mitochondria and cell cultures. These differences may be explained by several factors, first, the use of a mitochondria-enriched suspension in experiments on isolated mitochondria, while in cells the number of mitochondria is limited. Second, a highly hydrophobic Ven should primarily be localized in the lipid membrane, so that its effective concentration can vary greatly. And third, cell experiments typically permit one to perform very long-time incubation, which is impossible with isolated mitochondria. As follows from our data, TTFA eliminated the effect of not only Ven, but also other mPTP protectors, except for Cs, whose action also partially decreased. Presumably, this effect may be associated with several consequences of TTFA action, including electron transfer delay, activation of ROS production, and respiration decrease, the simultaneous occurrence of which can influence calcium accumulation and mPTP formation. Therefore, all these consequences may result in the loss of the protective activity of Ven. In addition, the difference in the effective concentrations of TTFA in relation to the substrates of various respiratory chain complexes, which are almost an order of magnitude higher for NAD-dependent substrates than for succinate, shows the dependence of the effect on the energetic state of mitochondria, which is significant in pathological cases. Presumably, the influence of Ven and TTFA can make itself evident in mitochondria with normal respiratory chain activity rather than in transformed mitochondria often encountered in cancer cells. Nevertheless, there is evidence indicating that, in Ven-resistant myeloma cells, mitochondrial respiration, oxidative phosphorylation, and the membrane potential are maintained at normal physiological levels, while Ven-sensitive cells show suppressed mitochondrial bioenergetics [34].

6. Conclusions

Thus, our data demonstrate that Ven exerts a protective effect on mPTP opening. This effect is related to its possible influence on ANT, a component of the pore-forming complex, and is similar to the effect of BA, which binds to ANT on the matrix side of ANT and inhibits mPTP opening. Their combined action, unlike other pore inhibitors (ADP, oligomycin), is not additive, indicating a common target. Besides, both act at comparable concentrations (50 μM for Ven and 20 μM for BA), and the effects of both are abolished by the ANT inhibitor CATR. Significant differences between them were observed in tests with the combined action with Cs, where BA strongly enhanced the effect of Cs, while Ven only weakly did. It can be assumed that in this case, BA and, to a lesser extent, Ven reduce the influence and ability of cyclophilin D to regulate mPTP opening. On the other hand, the high lipophilicity of Ven suggests its preferential distribution in the mitochondrial lipid membrane and subsequent influence on membrane proteins, including pore-forming components. Although Ven decreased the ROS production induced by TTFA, the effect of TTFA on the activation of pore opening prevailed, which confirms the dependence of the resistance to Ven on the state of the respiratory chain.

Abbreviations

ABC, ATP-binding cassette; ANT, adenine nucleotide translocase; AR, Amplex Red; BA, bongkrekic acid; CATR, carboxyatractyloside; Cs, cyclosporine A; FCCP, carbonyl cyanide-p-trifluoromethoxyphenylhydrazone; MCLA, 6-(4-methoxyphenyl)-2-methylimidazo[1,2-a]pyrazin-3(7H)-one hydrochloride; MDCL, MCLA-derived chemiluminescence; mPTP, mitochondrial permeability transition pore; MDR, multidrug resistance; TPP+, tetraphenylphosphonium ion; TTFA, 2-thenoyltrifluoroacetone; Ven, venetoclax.

Availability of Data and Materials

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

AK, TF, and NF designed the study; AK and NF performed experiments and wrote the manuscript on mitochondrial topic; TF wrote and edited the manuscript in the pharmaceutical aspect. All authors read and approved the final manuscript. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the AVMA guidelines for the Euthanasia of Animals: 2020 Edition and was reported in accordance with the ARRIVE guidelines. Manipulations were carried out by the certified staff of the Animal Department of the Institute of Theoretical and Experimental Biophysics and approved by the Commission on Biomedical Ethics of ITEB RAS (Protocol No. 01/2026 of 25 February 2026).

Acknowledgment

Not applicable.

Funding

The work has been supported by Russian government contract № 124020900031-0 of the Pirogov Russian National Research Medical University and the Russian government contract 075-00224-26-02 of the Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences.

Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBL55451.

References

[1] Morisaki T, Katano M. Mitochondria-targeting therapeutic strategies for overcoming chemoresistance and progression of cancer. Current Medicinal Chemistry. 2003; 10: 2517–2521. https://doi.org/10.2174/0929867033456431

[2] Mukherjee S, Bhatti GK, Chhabra R, Reddy PH, Bhatti JS. Targeting mitochondria as a potential therapeutic strategy against chemoresistance in cancer. Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie. 2023; 160: 114398. https://doi.org/10.1016/j.biopha.2023.114398

[3] Genovese I, Carinci M, Modesti L, Aguiari G, Pinton P, Giorgi C. Mitochondria: Insights into Crucial Features to Overcome Cancer Chemoresistance. International Journal of Molecular Sciences. 2021; 22: 4770. https://doi.org/10.3390/ijms22094770

[4] Winter JM, Yadav T, Rutter J. Stressed to death: Mitochondrial stress responses connect respiration and apoptosis in cancer. Molecular Cell. 2022; 82: 3321–3332. https://doi.org/10.1016/j.molcel.2022.07.012

[5] Goebel J, Chmielewski J, Hrycyna CA. The roles of the human ATP-binding cassette transporters P-glycoprotein and ABCG2 in multidrug resistance in cancer and at endogenous sites: future opportunities for structure-based drug design of inhibitors. Cancer Drug Resistance (alhambra, Calif.). 2021; 4: 784–804. https://doi.org/10.20517/cdr.2021.19

[6] Seeger MA, van Veen HW. Molecular basis of multidrug transport by ABC transporters. Biochimica Et Biophysica Acta. 2009; 1794: 725–737. https://doi.org/10.1016/j.bbapap.2008.12.004

[7] Xiao H, Zheng Y, Ma L, Tian L, Sun Q. Clinically-Relevant ABC Transporter for Anti-Cancer Drug Resistance. Frontiers in Pharmacology. 2021; 12: 648407. https://doi.org/10.3389/fphar.2021.648407

[8] Zhao L, Deng X, Li Y, Hu J, Xie L, Shi F, et al. Conformational change of adenine nucleotide translocase-1 mediates cisplatin resistance induced by EBV-LMP1. EMBO Molecular Medicine. 2021; 13: e14072. https://doi.org/10.15252/emmm.202114072

[9] Hu K, Lai Y, Zhou J, Hu C, Guo S, Zhang H, et al. Aberrant activation of adenine nucleotide translocase 3 promotes progression and chemoresistance in multiple myeloma dependent on PINK1 transport. International Journal of Biological Sciences. 2025; 21: 233–250. https://doi.org/10.7150/ijbs.101850

[10] Fedotcheva T, Shimanovsky N, Fedotcheva N. Involvement of Multidrug Resistance Modulators in the Regulation of the Mitochondrial Permeability Transition Pore. Membranes. 2022; 12: 890. https://doi.org/10.3390/membranes12090890

[11] Fedotcheva TA, Kruglov AG, Fedotcheva NI. Influence of Tariquidar, an ABC Transporter Inhibitor, on the Ca2+-Dependent Mitochondrial Permeability Transition Pore. Pharmaceuticals (basel, Switzerland). 2025; 18: 924. https://doi.org/10.3390/ph18060924

[12] Mukherjee N, Sheetz J, Shellman YG. Targeting the BCL2 Family: Advances and Challenges in BH3 Mimetic-Based Therapies. International Journal of Molecular Sciences. 2025; 26: 9859. https://doi.org/10.3390/ijms26209859

[13] Maffeo B, Panuzzo C, Moraca A, Cilloni D. A Leukemic Target with a Thousand Faces: The Mitochondria. International Journal of Molecular Sciences. 2023; 24: 13069. https://doi.org/10.3390/ijms241713069

[14] Roca-Portoles A, Rodriguez-Blanco G, Sumpton D, Cloix C, Mullin M, Mackay GM, et al. Venetoclax causes metabolic reprogramming independent of BCL-2 inhibition. Cell Death & Disease. 2020; 11: 616. https://doi.org/10.1038/s41419-020-02867-2

[15] Wang JQ, Li JY, Teng QX, Lei ZN, Ji N, Cui Q, et al. Venetoclax, a BCL-2 Inhibitor, Enhances the Efficacy of Chemotherapeutic Agents in Wild-Type ABCG2-Overexpression-Mediated MDR Cancer Cells. Cancers. 2020; 12: 466. https://doi.org/10.3390/cancers12020466

[16] Ibrahim MAA, Badr EAA, Abdelrahman AHM, Almansour NM, Shawky AM, Mekhemer GAH, et al. Prospective Drug Candidates as Human Multidrug Transporter ABCG2 Inhibitors: an In Silico Drug Discovery Study. Cell Biochemistry and Biophysics. 2021; 79: 189–200. https://doi.org/10.1007/s12013-021-00985-y

[17] Weiss J, Gajek T, Köhler B, Haefeli W. Venetoclax (ABT-199) Might Act as a Perpetrator in Pharmacokinetic Drug–Drug Interactions. Pharmaceutics. 2016; 8: 5. https://doi.org/10.3390/pharmaceutics8010005

[18] Liu J, Chen Y, Yu L, Yang L. Mechanisms of venetoclax resistance and solutions. Frontiers in Oncology. 2022; 12: 1005659. https://doi.org/10.3389/fonc.2022.1005659

[19] Bajpai R, Sharma A, Achreja A, Edgar CL, Wei C, Siddiqa AA, et al. Electron transport chain activity is a predictor and target for venetoclax sensitivity in multiple myeloma. Nature Communications. 2020; 11: 1228. https://doi.org/10.1038/s41467-020-15051-z

[20] Cartes-Saavedra B, Ghosh A, Hajnóczky G. The roles of mitochondria in global and local intracellular calcium signalling. Nature Reviews. Molecular Cell Biology. 2025; 26: 456–475. https://doi.org/10.1038/s41580-024-00820-1

[21] Bernardi P, Gerle C, Halestrap AP, Jonas EA, Karch J, Mnatsakanyan N, et al. Identity, structure, and function of the mitochondrial permeability transition pore: controversies, consensus, recent advances, and future directions. Cell Death and Differentiation. 2023; 30: 1869–1885. https://doi.org/10.1038/s41418-023-01187-0

[22] Kruglov AG, Nikiforova AB. The Switching of the Type of a ROS Signal from Mitochondria: The Role of Respiratory Substrates and Permeability Transition. Antioxidants (basel, Switzerland). 2024; 13: 1317. https://doi.org/10.3390/antiox13111317

[23] Towne V, Will M, Oswald B, Zhao Q. Complexities in horseradish peroxidase-catalyzed oxidation of dihydroxyphenoxazine derivatives: appropriate ranges for pH values and hydrogen peroxide concentrations in quantitative analysis. Analytical Biochemistry. 2004; 334: 290–296. https://doi.org/10.1016/j.ab.2004.07.037

[24] SUZUKI N, SUETSUNA K, MASHIKO S, YODA B, NOMOTO T, TOYA Y, et al. Reaction rates for the chemiluminescence of Cypridina luciferin analogues with superoxide: A quenching experiment with superoxide dismutase. Agricultural and Biological Chemistry. 1991; 55: 157–160. https://doi.org/10.1271/bbb1961.55.157

[25] Yamaguchi S, Kishikawa N, Ohyama K, Ohba Y, Kohno M, Masuda T, et al. Evaluation of chemiluminescence reagents for selective detection of reactive oxygen species. Analytica Chimica Acta. 2010; 665: 74–78. https://doi.org/10.1016/j.aca.2010.03.025

[26] Teranishi K, Hisamatsu M, Yamada T. Chemiluminescence of 2-methyl-6-arylimidazo [1, 2-a] pyrazin-3 (7H)-one in protic solvents: electron-donating substituent effect on the formation of the neutral singlet excited-state molecule. Luminescence. 1999; 14: 297–302. https://doi.org/10.1002/(SICI)1522-7243(199911/12)14:6<297::AID-BIO574>3.0.CO;2-Y

[27] Kruglov AG, Nikiforova AB, Shatalin YV, Shubina VV, Fisyuk AS, Akatov VS. Sulfur-containing compounds quench 3,7-dihydro-2-methyl-6-(4-methoxyphenyl)imidazol[1,2-a]pyrazine-3-one chemiluminescence: Discrimination between true antioxidants and quenchers using xanthine oxidase. Analytical Biochemistry. 2010; 406: 230–232. https://doi.org/10.1016/j.ab.2010.07.001

[28] Klener P, Sovilj D, Renesova N, Andera L. BH3 Mimetics in Hematologic Malignancies. International Journal of Molecular Sciences. 2021; 22: 10157. https://doi.org/10.3390/ijms221810157

[29] Garciaz S, Saillard C, Hicheri Y, Hospital MA, Vey N. Venetoclax in Acute Myeloid Leukemia: Molecular Basis, Evidences for Preclinical and Clinical Efficacy and Strategies to Target Resistance. Cancers. 2021; 13: 5608. https://doi.org/10.3390/cancers13225608

[30] Croce CM, Tait SWG, Garcia-Sáez AJ, Villunger A, Letai A, Walter HS, et al. What does BCL-2 do? From new molecular insights to the clinical implications. Cell Death and Differentiation. 2026; 33: 673–693. https://doi.org/10.1038/s41418-025-01607-3

[31] Liu D, Liu Z, Hu Y, Xiong W, Wang D, Zeng Z. MOMP: A critical event in cell death regulation and anticancer treatment. Biochimica Et Biophysica Acta. Reviews on Cancer. 2025; 1880: 189280. https://doi.org/10.1016/j.bbcan.2025.189280

[32] Morris JL, Gillet G, Prudent J, Popgeorgiev N. Bcl-2 Family of Proteins in the Control of Mitochondrial Calcium Signalling: An Old Chap with New Roles. International Journal of Molecular Sciences. 2021; 22: 3730. https://doi.org/10.3390/ijms22073730

[33] Sheth AI, Althoff MJ, Tolison H, Engel K, Amaya ML, Krug AE, et al. Targeting Acute Myeloid Leukemia Stem Cells through Perturbation of Mitochondrial Calcium. Cancer Discovery. 2024; 14: 1922–1939. https://doi.org/10.1158/2159-8290.CD-23-1145

[34] Palominos C, Fuentes-Retamal S, Salazar JP, Guzmán-Rivera D, Correa P, Mellado M, et al. Mitochondrial bioenergetics as a cell fate rheostat for responsive to Bcl-2 drugs: New cues for cancer chemotherapy. Cancer Letters. 2024; 594: 216965. https://doi.org/10.1016/j.canlet.2024.216965

[35] Haworth RA, Hunter DR. Control of the mitochondrial permeability transition pore by high-affinity ADP binding at the ADP/ATP translocase in permeabilized mitochondria. Journal of Bioenergetics and Biomembranes. 2000; 32: 91–96. https://doi.org/10.1023/a:1005568630151

[36] Zhao L, Tang M, Bode AM, Liao W, Cao Y. ANTs and cancer: Emerging pathogenesis, mechanisms, and perspectives. Biochimica Et Biophysica Acta. Reviews on Cancer. 2021; 1875: 188485. https://doi.org/10.1016/j.bbcan.2020.188485

[37] Lytovchenko O, Kunji ERS. Expression and putative role of mitochondrial transport proteins in cancer. Biochimica Et Biophysica Acta. Bioenergetics. 2017; 1858: 641–654. https://doi.org/10.1016/j.bbabio.2017.03.006

[38] Kharechkina ES, Nikiforova AB, Belosludtsev KN, Rokitskaya TI, Antonenko YN, Kruglov AG. Pioglitazone Is a Mild Carrier-Dependent Uncoupler of Oxidative Phosphorylation and a Modulator of Mitochondrial Permeability Transition. Pharmaceuticals (basel, Switzerland). 2021; 14: 1045. https://doi.org/10.3390/ph14101045

[39] Walter L, Miyoshi H, Leverve X, Bernard P, Fontaine E. Regulation of the mitochondrial permeability transition pore by ubiquinone analogs. A progress report. Free Radical Research. 2002; 36: 405–412. https://doi.org/10.1080/10715760290021252

[40] Fontaine E, Eriksson O, Ichas F, Bernardi P. Regulation of the permeability transition pore in skeletal muscle mitochondria. Modulation By electron flow through the respiratory chain complex i. The Journal of Biological Chemistry. 1998; 273: 12662–12668. https://doi.org/10.1074/jbc.273.20.12662

[41] Kruspig B, Valter K, Skender B, Zhivotovsky B, Gogvadze V. Targeting succinate:ubiquinone reductase potentiates the efficacy of anticancer therapy. Biochimica Et Biophysica Acta. 2016; 1863: 2065–2071. https://doi.org/10.1016/j.bbamcr.2016.04.026

[42] Dinu Iacob A, Confederat LG, Dragostin I, Morariu ID, Tutunaru D, Dragostin OM. Synergism of Synthetic Sulfonamides and Natural Antioxidants for the Management of Diabetes Mellitus Associated with Oxidative Stress. Current Issues in Molecular Biology. 2025; 47: 709. https://doi.org/10.3390/cimb47090709