Idebenone inhibits cell proliferation by blocking of ANO1/TMEM16A chloride channel in adenocarcinoma cells

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Abstract

Ca^{2+}-activated Cl^- channels (CaCCs) play a pivotal role in a number of physiological processes including regulation of cell proliferation, differentiation and apoptosis. The expression levels of anoctamin 1 (ANO1, TMEM16A), a CaCC, are significantly increased in several tumors such as prostate adenocarcinomas and head and neck squamous cell carcinomas. Recent studies revealed that inhibition of ANO1 significantly reduced cell proliferation, cell migration, tumorigenesis and cancer progression. Here, we performed cell-based screening of a collection of drugs and drug-like compounds to identify inhibitors of ANO1. We found that idebenone, a synthetic analog of coenzyme Q10, is a potent inhibitor of ANO1. Electrophysiological studies showed that idebenone completely blocked ANO1 activity in ANO1 expressing FRT cells and significantly inhibited current of CaCC in PC-3 cells expressing abundant endogenous ANO1. Idebenone strongly inhibited cell migration in PC-3 cells, and it did not affect the intracellular Ca^{2+} concentration. We investigated the effect of idebenone on the cell proliferation and apoptosis in adenocarcinoma cell lines. Idebenone significantly reduced cell proliferation and induced apoptosis in PC-3, CFPAC-1, HT-29, T-84 and Calu-3 cells having CaCCs activities. These data suggest that idebenone, an ANO1/CaCC inhibitor, has potential for use in cancer therapy.

Results

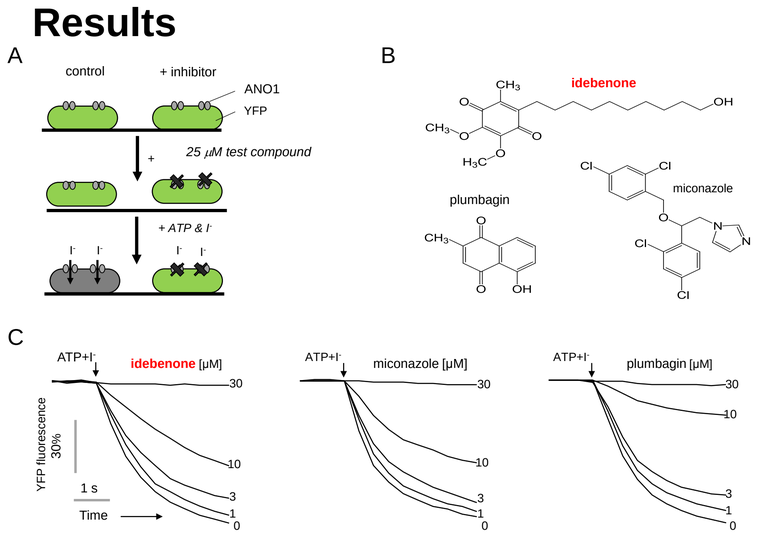

Figure 1. Identification of inhibitors of TMEM16A.

A) Principle of cell-based, fluorescence high-throughput screening assay. FRT cells stably expressing ANO1 and the YFP halide sensor were incubated for 20 min with test compound. Fluorescence was monitored in response to the addition of iodide and ATP. B) Chemical structures of ANO1 inhibitors. C) YFP fluorescence measured in single wells of 96-well plates, showing inhibitory effect of idebenone, miconazole and plumbagin on ANO1 channel activity. Indicated concentrations of idebenone, miconazole and plumbagin were added 20min prior to ANO1 activation by 100 μM ATP.

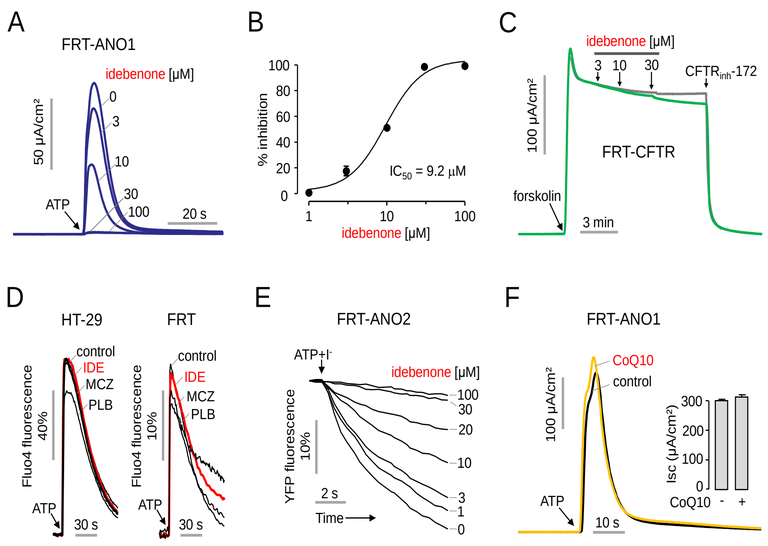

Figure 2. Characterization of idebenone, a ANO1/TMEM16A inhibitor.

A) Short circuit current measured in ANO1-expressing FRT cells in the presence of a transepithelial chloride gradient. Idebenone was added 10 min prior to ANO1 activation by 100 μM ATP. B) Summary of dose-response data from short circuit current measurement in FTR-ANO1 cells (mean ± S.E., n=3-4). C) Effect of idebenone on CFTR chloride channel activity was measured in FRT cells expressing human wild-type CFTR. CFTR was activated by 20 μM forskolin, a direct activator of adenylyl cyclase, and was inhibited by $CFTR_{inh}$-172, a specific CFTR inhibitor. D) Intracellular calcium concentration was measured by Fluo-4 fluorescence. HT-29 and FRT cells were pretreated for 20min with 30 μM idebenone (IDE), miconazole (MCZ) and plumbagin (PLB) and then CaCCs were activated by 100 μM ATP, a calcium agonist as indicated. E). YFP fluorescence of FRT cells stably expressing ANO2 (TMEM16B) and the YFP halide sensor was measured. The cells were incubated with indicated concentrations of idebenone for 20min and then ANO2 was activated by 100 μM ATP. F). Effect of Coenzyme Q10 (CoQ10) on ANO1 channel activity was observed in ANO1-expressing FRT cells. 100 μM CoQ10 was pretreated for 20min and then ANO1 was activated by 100 μM ATP. (left) Representative short-circuit current traces. (right) Summary of peak current (mean ± S.E., n=3-4).

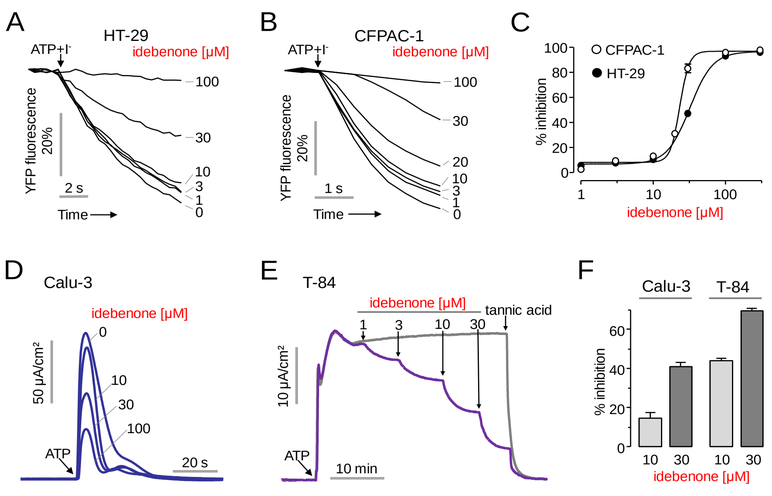

Figure 3. Inhibition of native CaCCs by idebenone in human epithelial cells.

A) Effect of idebenone on the activity of endogenous CaCCs was measured in HT-29 cells expressing the halide-sensitive mutant YFP. CaCCs were activated by 100 μM ATP.
B) Effect of idebenone on CaCCs was measured in CFPAC-1 cells expressing halide sensitive mutant YFP. C) Idebenone dose-response for inhibition of CaCCs in HT-29 (closed circles) and CFPAC-1 (open circles) cells. D) Representative traces of short circuit current measured in Calu-3 cells expressing endogenous CaCCs. Different concentrations of idebenone were pretreated for 20 minutes and then CaCCs were stimulated by 100 μM ATP. E) CaCCs current was stimulated by 100 μM ATP and then indicated concentration of idebenone was applied in T-84 cells. The remaining current was completely inhibited by 100 μM tannic acid. F) Summary of peak current inhibition with 10 and 30 μM idebenone in Calu-3 and T-84 cells.

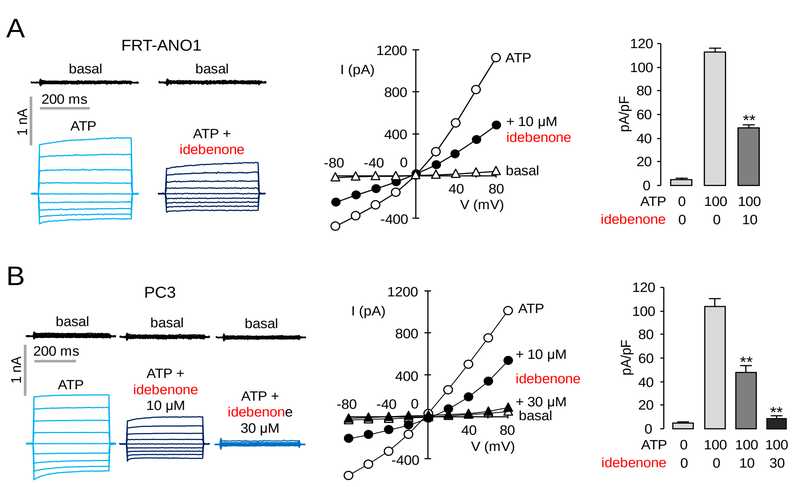

Figure 4. Idebenone inhibits CaCC chloride current in FRT-ANO1 and PC3 cells.

A) Whole cell ANO1 current recorded at a holding potential at 0 mV and pulsing to voltages between ± 80 mV (in steps of 20 mV) in the absence and presence of 10 μM idebenone (left). ANO1 was activated by 100 μM ATP. Current/voltage (I/V) plot of mean currents at the middle of each voltage pulse (center). The bar graphs summarize current density data measured at + 80 mV (mean ± S.E., n = 4). B) Whole cell patch clamp recordings of PC3 cells. CaCC current recorded in the absence and presence of idebenone (left). CaCC was stimulated by 100 μM ATP. Current/voltage (I/V) plot of mean currents at the middle of each voltage pulse (center). The bar graphs summarize current density data measured at + 80 mV (mean ± S.E., n = 4). **P < 0.001.

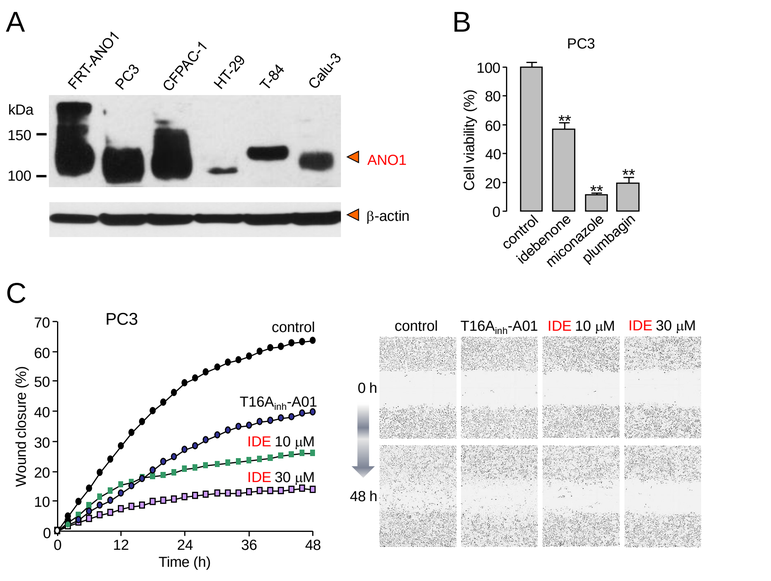

Figure 5. ANO1 expression in various adenocarcinoma cell lines and effect of ANO1 inhibitors on the cell viability.

A) Immunoblot of ANO1 protein in FRT-ANO1, PC3, CFPAC-1, HT-29, T-84, Calu-3 and A549 cells. Representatives of three sets of studies are shown. B) PC3 and A549 cells were treated with idebenone (30 μM), miconazole (30 μM) and plumbagin (30 μM), and cell proliferation was measured after 2 days using methanethiosulfonate (MTS) assays (mean ± S.E., n = 6). **P < 0.001. C) Wound healing assay in PC3 cells. Wound was performed after confluence and cells were treated with $T16A_{inh}$-A01 (30 μM) and idebenone (10 and 30 μM). (left) The wound closure was quantified at every 2 h post-wound using IncuCyte™ software package (mean ± S.E., n = 5). (right) Representative images taken at 0h and 48 h post wound (× 10).

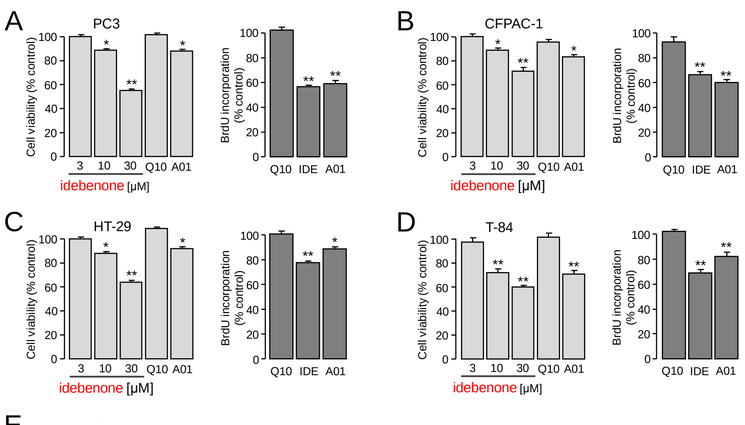

Figure 6. Effects of ANO1/CaCC inhibition on the proliferation of prostate, pancreas, colon and lung cancer cells.

A-F) PC3, CFPAC-1, HT-29, T-84 and Calu-3 cells were seeded in 96 well plates, and after 24 h incubation, treated with the indicated concentrations of idebenone (IDE), 100 μM coenzyme Q10 (Q10), 10 μM $T16A_{inh}$-A01 (A01) and 100 μM tannic acid (TA) in MTS assay. IDE (30 μM), coenzyme Q10 (100 μM), $T16A_{inh}$-A01 (10 μM) and TA (100 μM) were applied to the cells in BruU assay. Cell proliferation was estimated after 2 days via MTS (*left*) or BrdU (right) assay (mean ± S.E., n = 6). *P < 0.05, **P < 0.001.

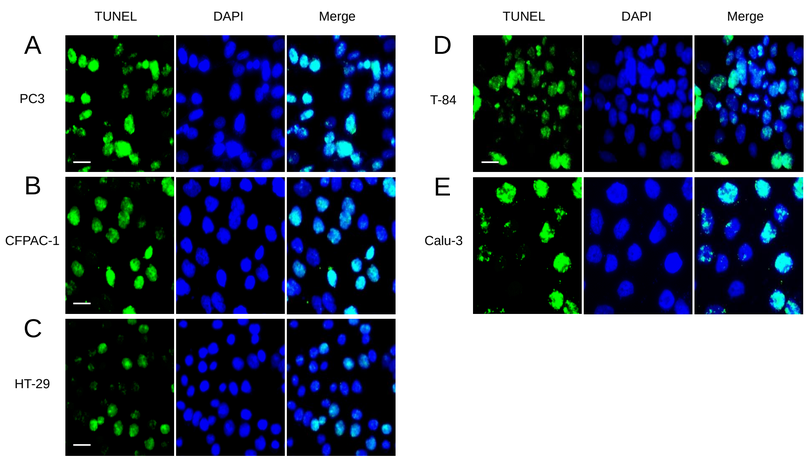

Figure 7. Idebenone induced apoptosis in prostate, pancreas, colon and lung cancer cells expressing ANO1/CaCCs.

A-F) PC3, CFPAC-1, HT-29, T-84 and Calu-3 cells were seeded in 96 well plates, and after 24 h incubation, treated with 30 μM idebenone. After 48 h of incubation, the cells were fixed and stained with TUNEL (terminal deoxynucleotide transferase-mediated dUTP nick-end labeling, green) and DAPI (4,6-diamidino-2-phenylindole, blue). Scale bars represent 20 μm.

Conclusion

To the best of our knowledge, this is the first work that shows idebenone, miconazole and plumbagin inhibit ANO1 chloride channel, and inhibition of ANO1 channel activity by these compounds may be, at least partially, involved in inhibition of cell proliferation..

This study shows that idebenone is a novel potent inhibitor of ANO1, and it did not affect intracellular calcium signaling and CFTR chloride channel activity. Idebenone potently blocked ANO1/CaCC channel activity in several adenocarcinoma cells including PC-3, CFPAC-1, HT-29, T-84 and Calu-3 cells, and significantly reduced cell proliferation, inhibited cell migration and induced apoptosis in these cells. These data suggest that idebenone may be a powerful candidate in the development of novel therapeutic agents for the treatment of cancer.