EFFECT OF MITOCHONDRIAL METABOLISM INTERFERING AGENTS (MMIAS) ON CANCER CELL MITOCHONDRIAL FUNCTION AND CHEMO-SENSITIVITY.

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Introduction

Mitochondria are vital organelles with their own genome, playing a key role in cell metabolism through oxidative phosphorylation. Apart from cell metabolism, mitochondrial function is directly linked to cell proliferation and apoptosis[1]. Thus, they constitute critical organelles for cell survival and worthy targets for the development of new anti-cancer agents as sensitizers to chemotherapy[2]. In this study, we investigated the effect of amiodarone, a class III anti-arrhythmic agent, which suppresses the respiratory chain at complex I and II[3] and metformin, an anti-hyper-glycemic drug, effective against the Type-II diabetes[4] which inhibits complex I of mitochondria on cancer cell mitochondrial function and the effect of these agents on cancer cells sensitisation to chemotherapy.

Materials and methods

Mitochondrial metabolism interfering agents (MMIAs)

To assess the effect of MMIAs on cell viability, titrations with various concentrations at different time points have been used based on a bibliographic study: a) metformin (Merck Co,USA) at a range of 50 to 400μM for 30,5h and b) amiodarone (Sanafi Aventis, Greece) at a range of 50 to 400μM for 8.5h. For chemo-sensitization experiments, a range of concentrations have been used for metformin and amiodarone with various concentrations of Cisplatin or Docetaxel.

AlamarBlue Assay®

The AlamarBlue® assay (ThermoScientific, USA) using the metabolic activity of cells to reduce resazurin (oxidized form; 7-hydroxy-3H-phenoxazin-3-1-10-oxide) to resorufin, counts the number of cells with active mitochondria. The resultant fluorescence of the reduced and the oxidized form, is measured at 590nm emission wavelength with an excitation at 530- 560nm. Cells in the appropriate culture medium were plated in a 96- well plate, at a concentration of 500cells/well. 10% v/v of AlamarBlue® was added in each well. As negative control (background measurement) culture medium without cells have been used. . In addition, Vitamin C has been as a positive control for full reduction of resazurin (5μl/well). Then, the relative fluorescence (in RFU units: fluorescence in each well minus background fluorescence) was measured every 30 min for 6.5 h, in a FLUOstar® Omega microplate reader (BMG LABTECH GmbH, Ortenberg, Germany).

CyQuant assay®

CyQUANT® Cell Proliferation Assay Kit (C7026; Invitrogen, UK) is based on the fluorescence of a dye bound to cellular nucleic acids. This assay provides a direct index of the DNA content of the viable, attached to the bottom of the plate wells, cells. Briefly, 500 cells per well in culture medium were plated in 96-well plates and titrated with various concentrations of the examined agents. The culture medium was removed and 50μl of the CyQUANT® reagent was added at the attached cancer cells. Fluorescence measurements have been obtained following a 45 min incubation time with a microplate reader (FLUOstar® Omega; BMG LABTECH GmbH, Ortenberg, Germany), at 480nm (excitation)and 520nm (emission).

Cell chemo- and radio-sensitivity experiments.

Cells were incubated with clinically established drugs; 8μM of Cisplatin and 0.62nM and 1.8nM of Docetaxel for 24h. Cell proliferation and survival experiments was performed using the Cyquant®.

Results

AlamarBlue® reduction by cancer cell lines after incubation with MMIAs.

Using Alamar Blue reduction it is observed that amiodarone suppressed the mitochondrial rezasurin reduction in a dose dependent manner in both cell lines while there is an intense, dose dependent, stimulatory effect on mitochondrial rezasurin reduction, as indicated by increased RFUs, was noted for all tested concentrations of metformin in both DU145 and A549 cell lines (Fig.1).

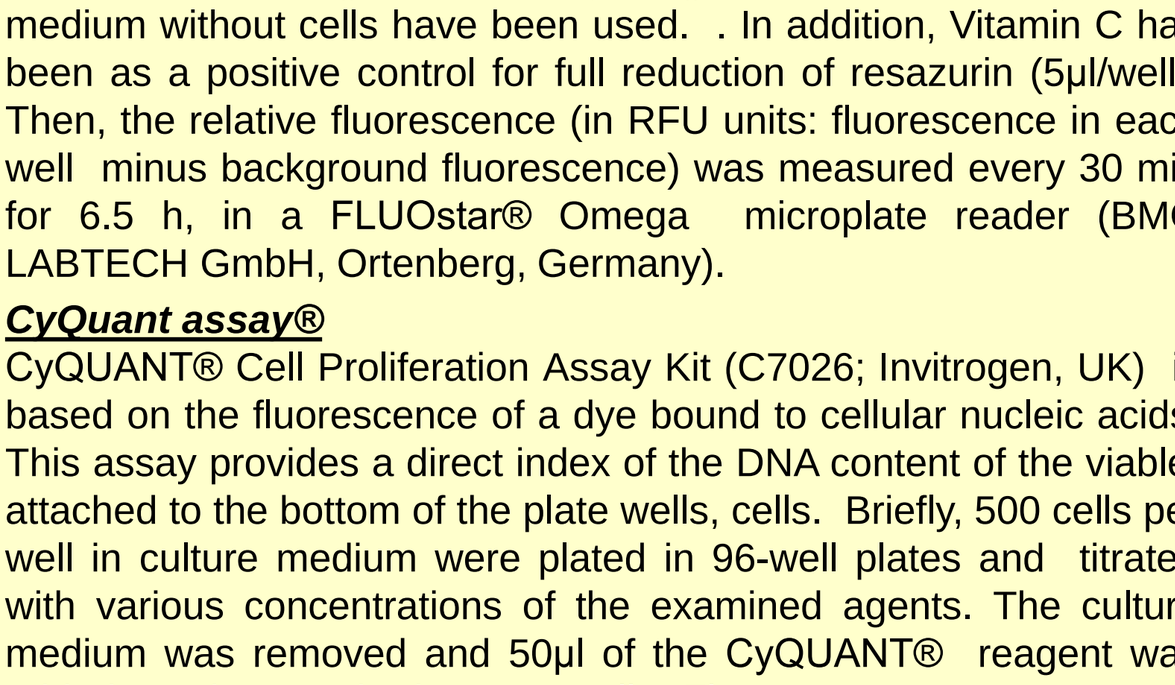

Figure 1. Relative fluorescence units (RFUs) obtained after incubation of DU145 and A549 cell lines with four different concentrations of amiodarone (2h incubation time) and metformin (24h incubation time). RFUs are recorded every 30 min for 6.5h

Chemosensitization with Metformin and Amiodarone

Co-incubation of amiodarone or metformin with cisplatin or docetaxel showed that although 100μM of amiodarone has a minor cytotoxic effect and treatment with 8μM of Cisplatin induced just the 20% reduction of the cell population in the two cells lines, simultaneous incubation with 100μM and 400μM of amiodarone and 8μM of Cisplatin resulted in synergistic effect between the two drugs. Moreover, combined incubation with amiodarone and docetaxel sensitizes to chemotherapy both cell lines (Fig.2).

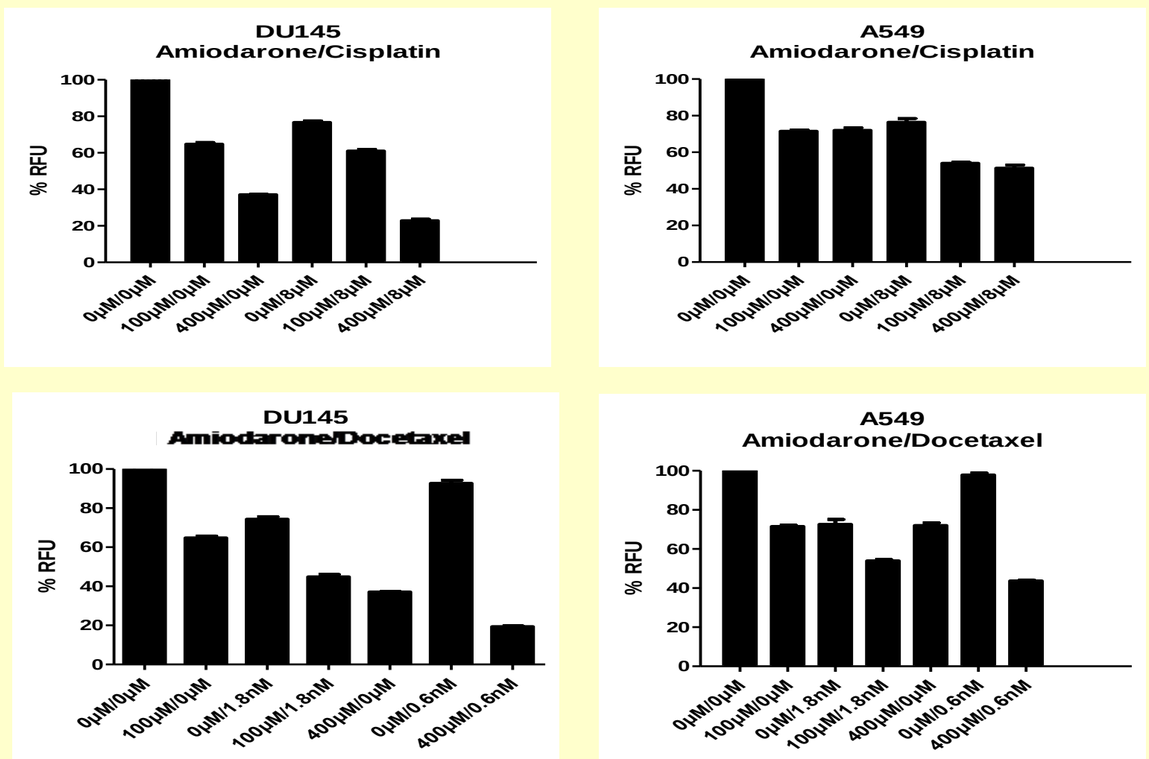

Figure 2. Chemosensitization experiments with amiodarone in DU145 and A549 cell lines, using the CyQuant assay.

Similar experiments with 400μM and 2000μM concentration of metformin (stimulatory and inhibitory of proliferation concentrations, respectively) showed clearly a sensitizing effect on both cell lines to cisplatin and to docetaxel (Fig.3).

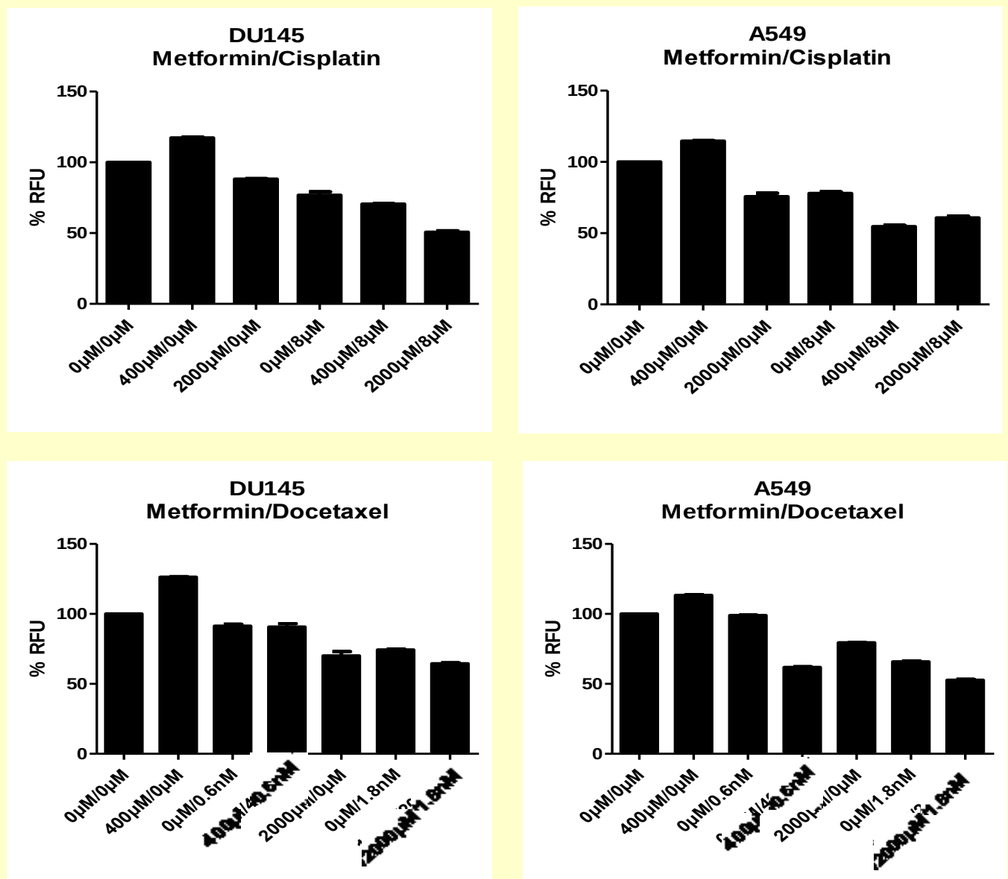

Figure 3. Chemosensitization experiments with metformin in DU145 and A549 cell lines, using the CyQuant assay.

Conclusion

Taking all the aforementioned into consideration we conclude that amiodarone and metformin affect mitochondrial function presumably by suppressing different mitochondrial complexes. Furthermore, it is clear that these two drugs which are used in non-cancer diseases may have anti-neoplastc effect in combination with chemotherapeutic drugs.

References

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