Evaluating CPA as a cytotoxic agent in endometrial and ovarian carcinoma cells

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Background

- Endometrial cancer (EC) and ovarian cancer (OC) are the most common and most deadly gynecological malignancies in the USA, respectively.[1]
- Initially responsive tumors of EC and OC become resistant to treatment.[2] The loss of epithelial cell-cell adhesions has been associated with chemoresistance.[3-5]
- Adenosine (Ado) is a tissue protective molecule that is produced in excess when cells are undergoing stress.[6,7]
- Our lab has recently shown that the activation of the adenosine A_1 receptor (A_1AR) induces promotes epithelial integrity in the endometrium.

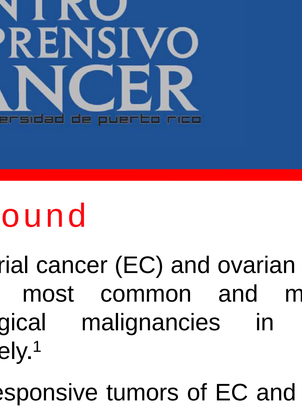

Figure 1. CD39 converts ATP and ADP into 5'-AMP, which is then phosphohydrolized into Ado by CD73. Ado then binds to ADORA1.[7] Figure has been modified from (8).

- We initially hypothesized an A_1AR agonist combined with chemotherapy regimen paclitaxel may chemosensitize EC and OC cells (Figure 2).

Background and Rationale

Figure 2. MTT assays show combination CPA + paclitaxel reduces HEC-50, KLE, and HEYA8 cell viability. CPA alone accounts for a significant change in cell viability, suggesting CPA as a single-agent may be cytotoxic.

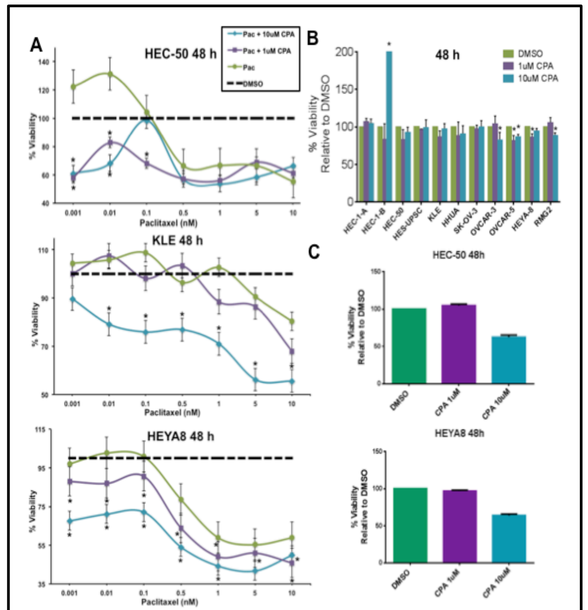

Figure 2. Cells were treated for 48 h and cell viability assessed by MTT assays. **(A)** CPA chemosensitizes HEC-50, KLE, and HEYA8 to paclitaxel. Cells were treated with paclitaxel with or without CPA at concentrations as indicated. Values are presented as mean percentage ± SEM and are normalized to DMSO cells. Graphs represent a single experiment performed in quadruplicates. **(B)** and **(C)** show the effect of CPA alone on cell viability. Cells were treated with 1 μM or 10 μM CPA, or DMSO. Values are presented as mean percentage ± SEM and have been normalized to DMSO. Graphs **(C)** are representative of three independent studies of 32 wells/per treatment group. * indicates $P < 0.05$ by ANOVA.

Hypothesis

- CPA is affecting cell viability in EC and OC cells by inducing either cell cycle arrest or cell death.

Methods

- Cell Cycle analysis by PI staining/FACS
- Cell Death analysis by Annexin V-PI/FACS
- Cell Viability analysis by Trypan Blue staining

Results

Figure 3. CPA does not induce cell cycle arrest.

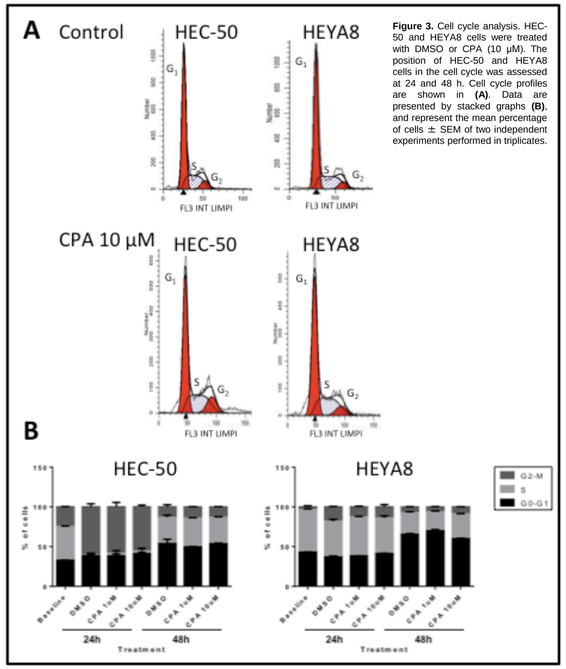

Figure 3. Cell cycle analysis. HEC-50 and HEYA8 cells were treated with DMSO or CPA (10 μM). The position of HEC-50 and HEYA8 cells in the cell cycle was assessed at 24 and 48 h. Cell cycle profiles are shown in **(A)**. Data are presented by stacked graphs **(B)**, and represent the mean percentage of cells ± SEM of two independent experiments performed in triplicates.

Figure 5. Cell viability is not found altered by CPA when assessed by Trypan Blue staining. An insignificant number of cells detached from the cell plates.

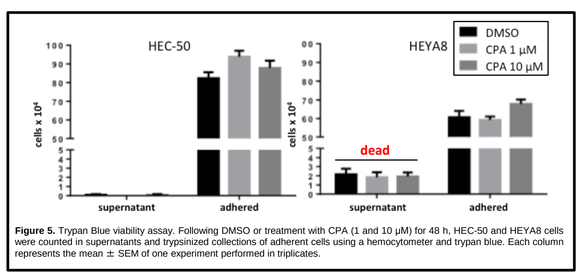

Figure 5. Trypan Blue viability assay. Following DMSO or treatment with CPA (1 and 10 μM) for 48 h, HEC-50 and HEYA8 cells were counted in supernatants and trypsinized collections of adherent cells using a hemocytometer and trypan blue. Each column represents the mean ± SEM of one experiment performed in triplicates.

Results

Figure 4. CPA does not induce cell death.

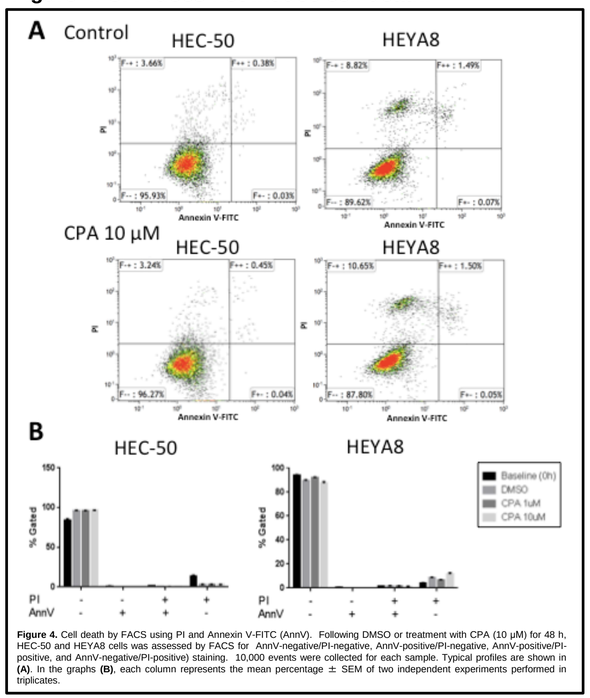

Figure 4. Cell death by FACS using PI and Annexin V-FITC (AnnV). Following DMSO or treatment with CPA (10 μM) for 48 h, HEC-50 and HEYA8 cells was assessed by FACS for AnnV-negative/PI-negative, AnnV-positive/PI-negative, AnnV-positive/PI-positive, and AnnV-negative/PI-positive) staining. 10,000 events were collected for each sample. Typical profiles are shown in **(A)**. In the graphs **(B)**, each column represents the mean percentage ± SEM of two independent experiments performed in triplicates.

Conclusions

- The reduction in cell viability with CPA, as seen by MTT assays, was not due to cell cycle arrest or cell death.
- Trypan Blue viability studies indicate the effects seen with MTT assays may be due to CPA downregulating MTT-reducing enzymes or its weakening of focal adhesions.
- Future studies will address these possibilities.

References

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