Ivermectin synergizes with interferon-gamma to overcome resistance in renal cancer

Cheyenne J. Roberts[1], Maria Jose Cuevas[2], Dr. Arnold H. Zea[3,4]

[1]University of California, Los Angeles, CA, [2]University Javeriana, Cali, Colombia, [3]S. Stanley Cancer Center, [4]Department of Microbiology, LSUHSC, New Orleans, LA

Introduction

- Renal cell carcinoma (RCC) treatment challenges mostly due to tumor heterogeneity and resistance to immune-based therapies.
- Interferon-gamma (IFN-γ) has been shown in Dr. Zea's lab to induce tumor cell death and growth arrest in some RCC tumors, primarily through increased production of nitric oxide (NO). However, some tumors remain resistant to IFN-γ treatment most probably regulated by the tumor micro-environment.
- IVM, is an FDA-approved antiparasitic drug first introduced in the 1970s, that has recently gained attention for its emerging anti-cancer and immunomodulatory properties including overcoming resistance.
- We are interested in using ivermectin (IVM) to modulate NO production, enhancing its anti-tumor effects while preventing chronic overproduction that may drive resistance.
- Therefore, IFN-γ is an anti-tumor agent and IVM could overcome tumor resistance in RCC, and their combination could conceivably enhance the effectiveness of immunotherapy (***Figure 1***).

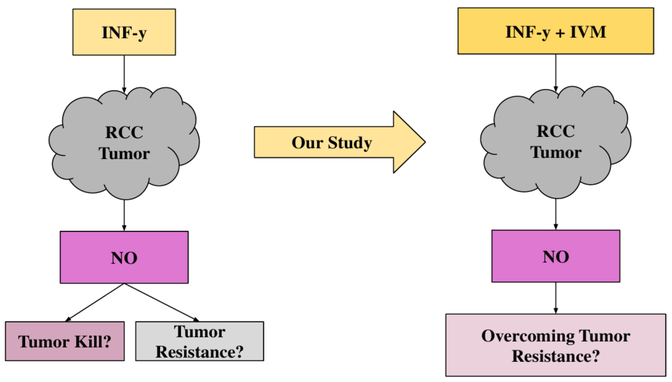

Figure 1

Objectives

- The objective of this study is to investigate the potential anti-tumor activity of IVM alone or in combination with IFN-γ to overcome tumor resistance mediated by IFN-γ.
- The effect of NO production and its association with anti-tumor activity will be also assessed.

Hypothesis

- We hypothesize that ivermectin can reduce tumor cell proliferation and enhance the therapeutic effects of IFN-γ in resistant renal cell carcinoma (RCC) cell lines.

Methods

- Four different murine RCC cell lines (R0, R1, R2 and CL19) with different responses to IFN-γ were used.
- 40,000 cells from each cell line were plated in 24 well plates, in RPMI plus 10% fetal bovine serum.
- The cells were stimulated with 10 and 100 U/μL of IFN-γ and with increasing concentrations of IVM at 2.5, 5, 10, 15, and 20 μM alone or in combination.
- The cells were incubated for 24, 48, and 72 hours after stimulation.
- At each time-point, supernatants were collected to measure NO (Greiss Assay) and IFN-γ levels
- Cell growth was assessed by MTT assay.
- Unstimulated cells were used as controls and all experiments were repeated twice.
- Data analysis was measured by GraphPad software Prism 5.0. The non-parametric Student t-test was used. P< 0.05 were significant.

Nitrite and cell growth base-line

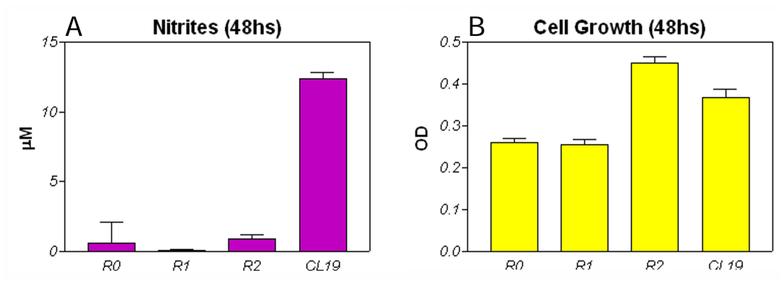

Figure 2. (A) At base line, Nitrite production was significantly more abundant in CL19 as compared to the other cell lines (10-fold). (B) R2 and CL19 cells grew faster than R0 and R1 at 48hrs (63%). The figure is representative of two different experiments ± SEM.

INF-γ stimulation

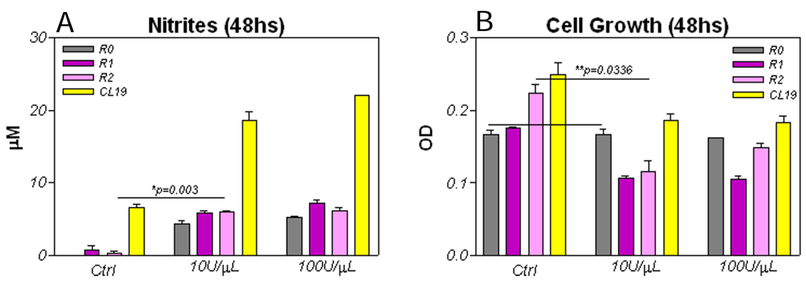

Figure 3. (A) All cell lines showed an increased production of NO after 48hs treatment with 10 U/μL of IFN-γ compared to un-stimulated cells*P=0.003. (B) Despite nitrite production, R1, R2, and CL19 cell lines showed decrease cell proliferation **P=0.0336, whereas R0 was resistant to IFN-γ stimulation. The graphs are representative of two different experiments, ± SEM.

Ivermectin (IVM)

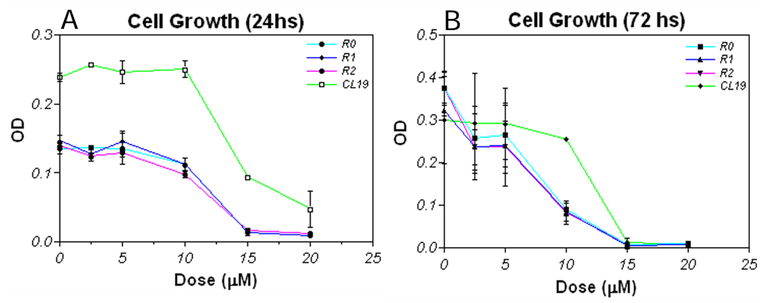

Figure 3. (A) IVM has no effect on cell growth at 24 and 48hrs. The $IC_{50}$was achieved at 7.5 μM IVM concentration. (B) IVM alone inhibits cell growth in R1, R2 and CL19, however, R0 shows some signs of resistance to IVM at 72 hrs. IVM has no effect on nitrite production. These experiments were repeated twice, and data is presented as mean ± SEM.

IVM + INF-γ Combination

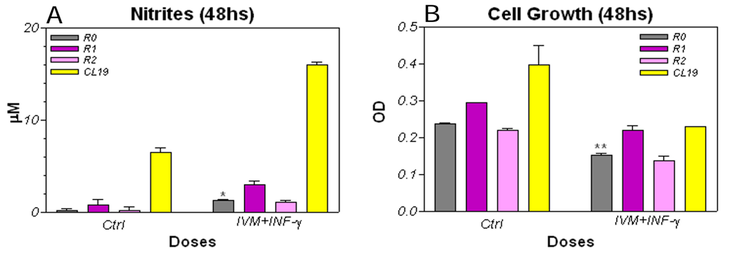

Figure 4. (A) After combo treatment, R0 showed an increased nitrite production with the combo treatment (*P=0.0348). (B) A significant **P=0.004 decrease in R0 growth was observed at 48 hrs. Indicating that a synergistic effect occurs, overcoming R0 resistance. The figure is representative of two different experiments ± SEM.

Conclusions

- These results suggest that ivermectin may serve as a promising therapeutic agent for reversing resistance to immunotherapy in kidney cancer, particularly in contexts where IFN-γ contributes to anti-tumor activity.
- The combination of IVM with IFN-γ could enhance the efficacy of existing treatments. However, more research is needed to understand the IVM mechanisms involved in overcoming resistance in humans.

Future Plans

- Investigate the specific mechanisms and signaling pathways associated with the use of IVM in combination with IFN-γ-induced responses.
- Explore IVM's impact on the tumor microenvironment inhibiting tumor growth in animal models.
- Validation of the IVM effectiveness needs to have more research, to allow these findings to be translated into clinical trials.

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