Synergistic Combination of Vasculature Disrupting Agent with TLR7/8 Agonist: Promising Strategy for Melanoma Therapy

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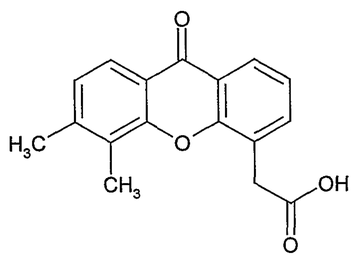
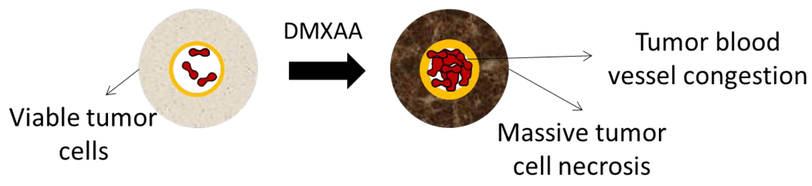
ABSTRACT

Gardiquimod is an imidazoquinoline compound and is a potent toll-like receptor 7 and 8 (TLR7/8) agonist. It causes activation of innate immune response and is known to have potent anti-viral and anti-tumor effect [1, 2]. It activates NFκB and MAP kinase pathways in innate immune cells and is speculated to stimulate antigen presenting cells (APCs) which were rendered tolerant in immuno-suppressed tumor microenvironment. 5,6-Dimethylxanthenone- 4-acetic Acid (DMXAA) exerts its anti-tumor effect by disrupting the tumor vasculature leading to generation of a necrotic center in a solid tumor. However, the limitation with DMXAA treatment is that the tumor cells present in the periphery are unaffected by the drug, leading to incomplete therapy. In this research, combination of gardiquimod with DMXAA was assessed to target B16 melanoma in a mouse model. Uniform and spherical gardiquimod encapsulated PLGA nanoparticles were prepared using single emulsion method. Their size was ~193 nm and the encapsulation efficiency was found to be 11.4 µg/mg. The role of nanoparticulate formulation was assessed by observing improved activation of BMDCs in the presence of PLGA-gardiquimod as compared to free gardiquimod. The nanoparticle uptake by BMDCs was also confirmed by fluorescence imaging. Further, PLGA-gardiquimod and DMXAA in the ratio of 1:10, 1:100, 1:200 and 1:500 synergistically enhanced cytokine (TNFα and IL-12) secretion from BMDCs. Mice treated with the combination had significantly lower tumor volume and a higher survival rate as compared to control groups, which was also in good correlation with immuno-chemical analyses from tumor tissue samples. This research highlights combination of vasculature disrupting with immuno-stimulation as a promising approach for management of solid tumor.

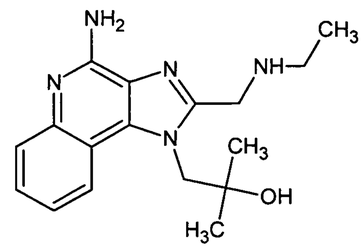
INTRODUCTION



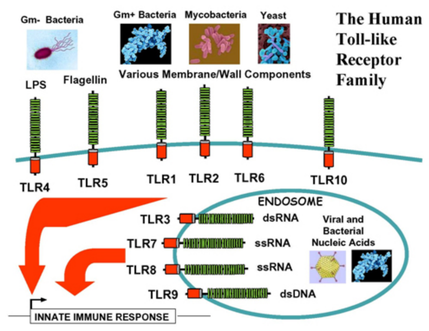


Cross-section of tumor blood vessel

Vasculature Disrupting Agent: 5,6-dimethylxanthenone 4-acetic acid (DMXAA)

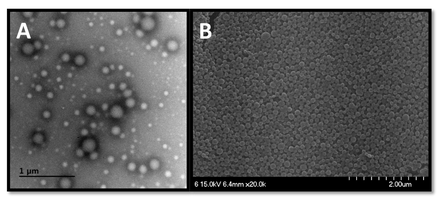


TLR7/8 agonist : Gardiquimod



MATERIAL CHARACTERIZATION

- Gardiquimod encapsulated PLGA nanoparticles were prepared by single emulsion method

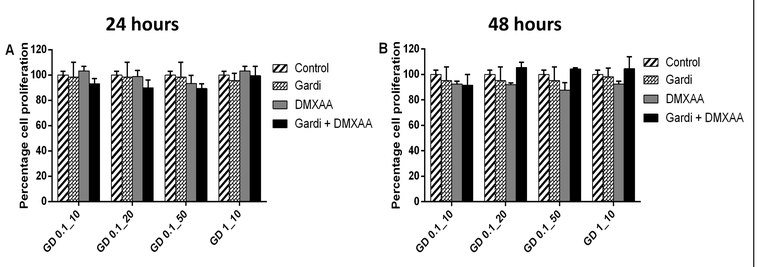


(A) Transmission electron micrographs and **(B)** Scanning electron micrographs of gardiquimod encapsulated PLGA nanoparticles.

Size	193.9 ± 49.9 nm
Encapsulation efficiency	11.1 ± 0.3 µg/mg

Table: Size and encapsulation efficiency of gardiquimod encapsulated PLGA nanoparticles.

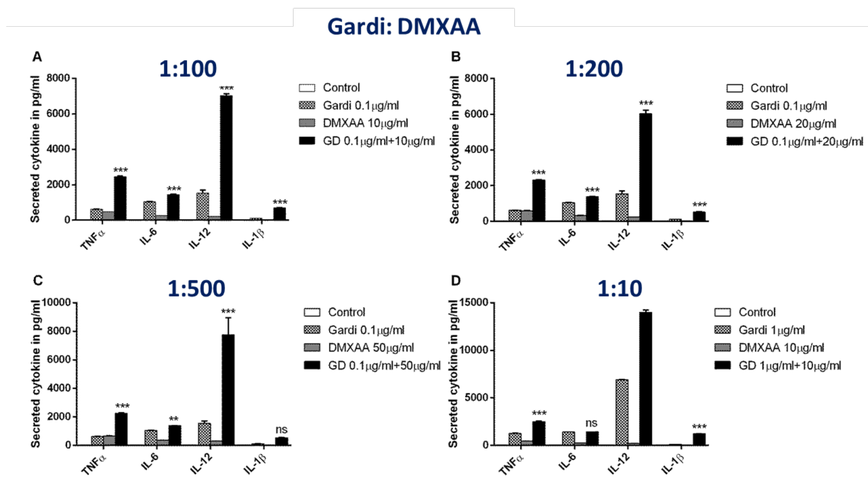
- Gardiquimod encapsulated PLGA nanoparticles along with DMXAA were tested for their toxicity against B-16 melanoma cells



Percentage proliferation of B-16-F10 melanoma cells after **(A)** 24 hours and **(B)** 48 hours of treatment with gardiquimod, DMXAA and combination at various concentrations.

RESULTS : *In vitro*

Cytokine (TNFα, IL-6, IL-12 and IL-1β) secretion levels of BMDCs after treatment with gardiquimod, DMXAA and combination at various concentrations
(A) gardiquimod 0.1 µg/ml + DMXAA 10 µg/ml, **(B)** gardiquimod 0.1 µg/ml + DMXAA 20 µg/ml, **(C)** gardiquimod 0.1 µg/ml + DMXAA 50 µg/ml, **(D)** gardiquimod 1 µg/ml + DMXAA 10 µg/ml. ***p<0.001 by one-way ANOVA test.



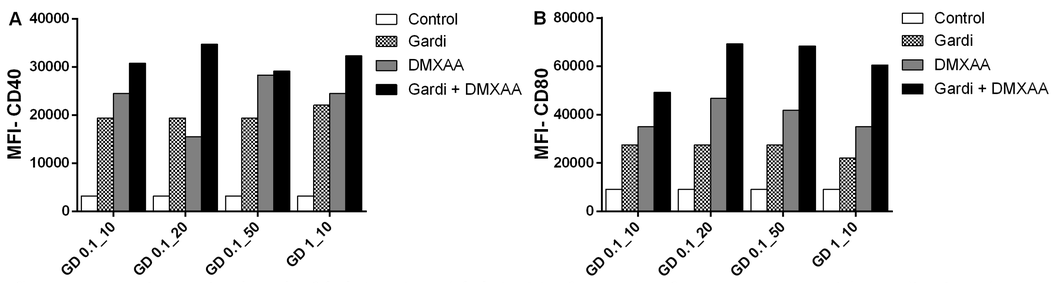
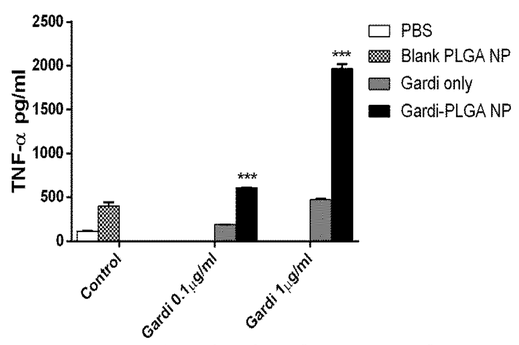
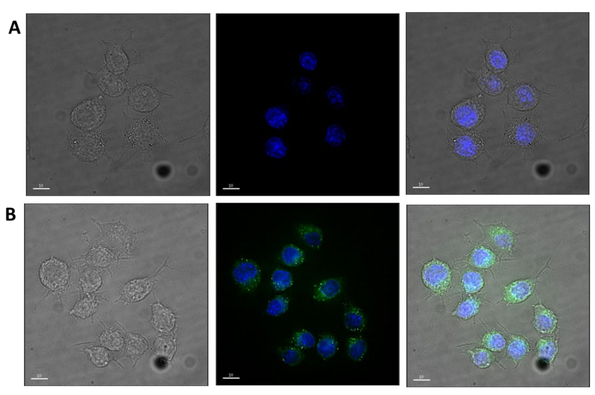


Figure 3 Mean fluorescence intensity (MFI) of **(A)** CD40 and **(B)** CD80 of BMDCs after treatment with gardiquimod, DMXAA and combination at various concentrations.

- Role of nanoparticulate formulation of gardiquimod on DCs



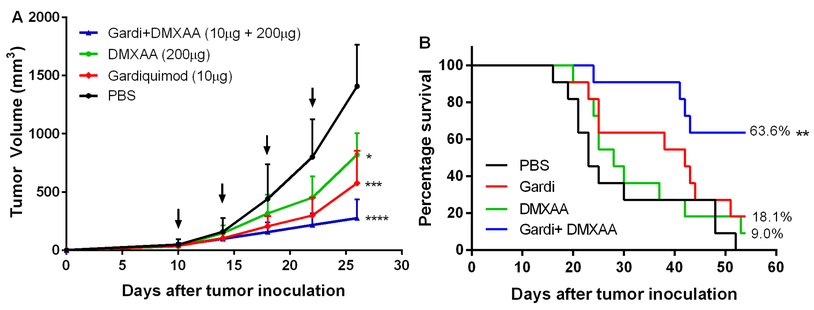
Cytokine TNFα secretion levels of BMDCs after treatment with blank PLGA nanoparticles, gardiquimod only and gardiquimod encapsulated PLGA nanoparticles ***p<0.001 by one-way ANOVA Test, Bonferroni's post test.

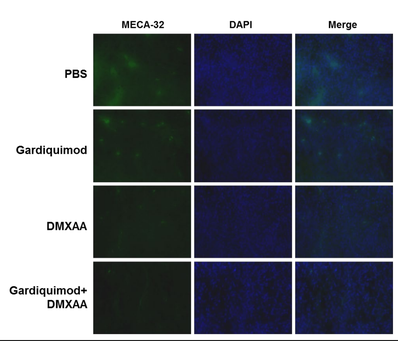


Nanoparticle uptake by cells was confirmed by fluorescence imaging of DC2.4 cells after treatment with ICG and gardiquimod encapsulated PLGA nanoparticles. Bright field, nuclear stained (dapi, blue), and merged images (left to right) of **(A)** control cells and **(B)** ICG labelled nanoparticle (green) treated cells.

RESULTS : *In vivo*

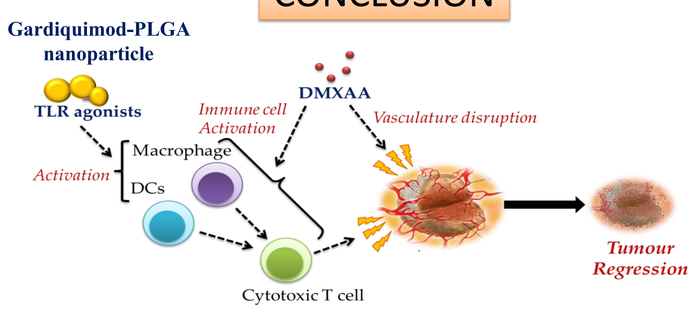
(A) Tumor volume ratio with respect to day 1 until day 27 in C57BL/6 mice immunized with Gardiquimod, DMXAA and combination. Injection time points indicated by black arrows. *p<0.05, ***p<0.001, ****p<0.0001 by one-way ANOVA test, Bonferroni's post test, n=8 **(B)** Kaplan Meier survival plot of immunized mice ** p=0.0024, n=11.





Immunohistochemical images of tumor sections for various treatment groups labelled with (left to right): endothelial marker (MECA-32, green), nuclear stain (dapi, blue), and merged at day 14 after tumor inoculation.

CONCLUSION



The synergy in the combination of DMXAA and gardiquimod mainly works at two levels. Firstly, synergistic immuno-stimulatory effect is observed in dendritic cells. Secondly, tumor inhibition is speculated to be achieved by targeting the tumor center by vasculature disruption and tumor periphery mainly by the immune cells.

REFERENCES

[1] F. Ma, J. Zhang, J. Zhang, C. Zhang, Cell Mol Immunol, 7 (2010) 381-388.
[2] M. Buitendijk, S.K. Eszterhas, A.L. Howell, AIDS Research and Human Retroviruses, 29 (2013) 907-918.

ACKNOWLEDGEMENT

This work was supported by the grant from Korea Basic Science Institute (D36401, KSH), the R&D Convergence Program (CRC-15-02-KRIBB, KSH) of NST (National Research Council of Science & Technology) of Republic of Korea, and the National Research Foundation of Korea (NRF) grant (2014R1A2A1A10049960, YTL) funded by the Korean government (MEST).