

Impact of Vitamin C on Genistein induced apoptosis during treatment of prostate cancer cells

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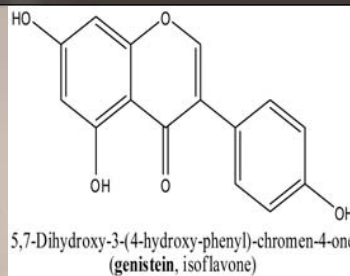


ABSTRACT

This study was designed to define the impact of vitamin C on genistein apoptosis using the methods adapted from the MTT assay. Reactive Oxygen Species (ROS) can promote cancer cell proliferation when they are at elevated levels. Vitamin C is a water-soluble antioxidant capable of inhibiting the formation of ROS. Genistein, an isoflavone found in plants, also possesses the ability to inhibit ROS formation. The combination of genistein and vitamin C was more efficient in tumor suppression than when the drugs were given separately. Although the combined treatment allows the cancer cell to grow initially trying to avoid side effect, the treatment was eventually more enhanced than the single treatment of vitamin C. When we compared this to our previous studies of treating cancer cells with genistein only, we find out that the combination treatment is more efficacious. This study suggests that treatment of prostate cancer using genistein can be enhanced by adjuvant treatment with vitamin C.

INTRODUCTION

Prostate Cancer (PCa) is the most common non-skin malignancy in men and is a rising health problem world-wide. Recent evidence gathered in our laboratory suggests that treatment of prostate cancer with genistein has serious side effect of necrosis on the cancer cells. In order to reduce this side effect, we use vitamin C as an additive to genistein therefore decreasing cancer cells death through necrosis but increasing its death by apoptosis. This will allow for reduction in pain and loss of hair that prostate cancer patients experience in metastasize and advance cancer..



HYPOTHESIS

It is hypothesized that treating prostate cancer with vitamin C as an adjuvant will enhance the recovery of the cells. We also hypothesized that combined treatment of vitamin C and genistein will give a better enhancement compared to single treatment of genistein.

OBJECTIVES

To determine the potential therapeutic additive effect of vitamin C in combination with genistein during treatment of prostate cancer

MATERIALS AND METHODS

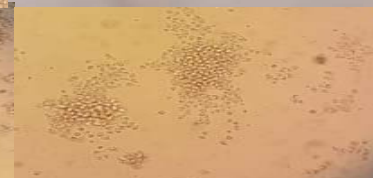
- 1 x 10⁶ cell LNCaP cells was cultured on RPMI 1640 growth media in an incubator until they reach their log phase (24-60hrs) at this phase they are counted using Trypan Blue Assay
- Cells were transferred to a 48 well ELISA plate, where they are cultured once again to reach their log phase.
- The cells were then treated with vitamin C, genistein and a combination of vitamin C and genistein in different treatment groups such that the concentration of each treatment ranges from 10uM, 20uM, 30uM, 40uM, 50uM and 70uM.
- For the combined treatment a constant vitamin C concentration of 30uM was used along side varying concentration of genistein 10uM to 70uM.
- After 24hrs treatment period the cells are analysed for viability using the MTT assay and ELISA plate reader
- The absorbance readings from the ELISA plate was converted to percentage and plotted on the Y-axis against the concentration of each treatment drug on the x-axis
- Statistical analysis was used to confirm significance difference in each treatment group.



24hrs incubation after treatment with 40uM genistein showing the beginning of apoptosis



24hrs incubation after treatment with vitamin C showing the beginning of apoptosis



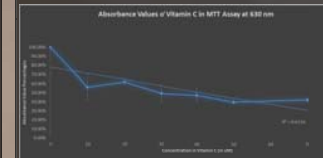
24hrs incubation after combined treatment with a combination of 30uM vitamin C and 40uM of genistein

CONCLUSION

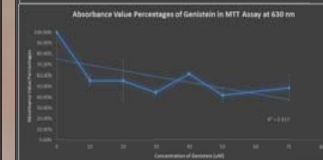
Induced apoptosis is one of the pathway that vitamin C and/ or genistein exert their cytotoxic effects. The significance of this is that vitamin C can be used as adjuvant in chemotherapy. Through induced apoptosis, vitamin C and genistein can kill cancer cells in a manner that is much less deleterious to the surrounding cells. Thus, these limit the damage done to the surrounding cells of the body through necrosis.

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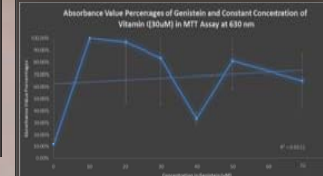
RESULTS



A downward trend can be observed from the graph. This graph shows that increasing vitamin C concentration does have a cytotoxic effect on LNCaP cancer cell population.



This graph shows that genistein does decrease LNCaP cancer cell population up to a concentration of 30uM. A spike in resistance from LNCaP cancer cells appears at a concentration of 40 uM followed by a decrease and leveling of the number of LNCaP cells.



This graph shows a sharp increase in LNCaP cells absorbance initially at 10 uM, followed by a sudden decrease as genistein concentration approaches 40 uM. The LNCaP cells show resistance as the genistein concentration approaches 50 uM, followed by a decrease in the number of LNCaP cells.

DISCUSSION

The preliminary data support the hypothesis. While Vitamin C treatment exhibited an immediate decrease in cell population, the combined treatment of vitamin C and genistein actually showed an increase in cell proliferation after addition of 10 uM of genistein. This is because the bi-phasic nature of genistein was impacted on the treatment group. A decrease in the population of LNCaP cells was then proceeded by this spike.

REFERENCES

- Joseph MA, Moysich KB, Freudenheim JL, Shields PG, Bowman ED, et al.: Cruciferous vegetables, genetic polymorphisms in glutathione StransferasesM1andT1, and prostate cancer risk. *NutrCancer* 50,206–213, 2004.
- Ambrosini GL, de Klerk NH, Fritschi L, Mackerras D, and Musk B: Fruit, vegetable, vitamin A intakes, and prostate cancer risk. *Prostate Cancer Prostatic Dis* 11, 61–66, 2008.