Possible Effects of Ursolic Acid on the Infectivity of the Simian Virus 40 in Vero cells

Tiana Elkins, Lisa Smith, and Karsten Hueffer, Department of Biology and Wildlife, University of Alaska, Fairbanks, Fairbanks, Alaska

Introduction

The effects of ursolic acid on the infectivity of the simian virus 40 (SV40), which enter the cell through lipid-rafts (microdomains of the plasma membrane and are rich in cholesterol, as well as glycosphingolipids) (Duncan et al, 2002.) will be explored. Ursolic Acid is a phytosterol as well as a triterpenoid found in fruits such as apples, blueberries, cranberries, and prunes. It has been demonstrated that UA has anti-inflammatory, antitumor, and antimicrobial properties. (Top Culture, date unknown.)

There have been no published studies dealing with ursolic acid and the SV40 virus; however, there are studies on the effects of ursolic acid on other viruses and tumorgenicity. In one study, ursolic acid is shown to reduce the growth of cancerous cells. (Wu et al, 2011) In another study, ursolic acid is shown to have an antiviral effect on the human papilloma virus and the influenza strain H1N1. (Kazakova et al, 2010.) Unpublished data has shown that ursolic acid can alter the structure of lipid-rafts; it modifies them to be smaller.

Vero cells will be infected with SV40 virus and analyzed with fluorescent microscopy to quantify how many cells are infected after treatment with varying concentrations of ursolic acid. The objective of this study is to determine if ursolic acid has the ability to reduce the infectivity of the SV40 virus.

Materials and Methods

Cells, Virus, Antibodies, and Reagents:

The African green monkey kidney epithelial cell line Vero was cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Newborn Calf Serum (NCS) and 1% Penicillin: streptomycin at 37°C, 6% CO_2. SV40 virus was obtained from ATCC. Mouse monoclonal antibodies against T-antigen were obtained from Abcam Inc, and Goat-anti-mouse Alexa Fluor 488 conjugated secondary antibody was purchased from Invitrogen (Carlsbad, CA). 90% Ursolic Acid was dissolved in dimethyl sulfoxide (DMSO) and diluted to final concentrations in DMEM. The final concentration of DMSO in prepared solutions was no greater than 0.6%.

Infecting the Cells:

Vero cells were treated with 0μM, 5μM, 20μM, 50μM, and 60μM of ursolic acid for 1 hour, then infected with SV40 for 1 hr. Cells are washed three times with HBSS media and replenished with media containing ursolic acid, and fixed with 4% paraformaldehyde after 24 hours of incubation.

T-Antigen Stain:

Antibody against the viral Large T-antigen is diluted 1:500 in a 1% Triton X/BSA solution. Cells are incubated in primary antibody for 1 hour at room temperature. The cells are then incubated in the fluorescently tagged secondary antibody at 1:1000 dilution in 1%TritonX/BSA for 15 minutes. The cover slips are washed after each incubation three times.

DAP-I Stain:

The DAP-I stain allows the detection of individual cells. DAP-I stains the DNA. The DAP-I is diluted 1:100 using PBS. The infected cover slips are then incubated for 15 minutes at room temperature, washed three times, and mounted to slides using ProLong Antifade Gold Reagent, and are left in a dark drawer for 24 hours.

Getting the Results:

The cells were counted by computer and hand, and averaged out. The numbers of un-infected and infected cells were determined. The standard deviation was taken for each average. One-way ANOVA was used to determine that the p-value was .72, making the differences in infectivity insignificant.

Infected Cells

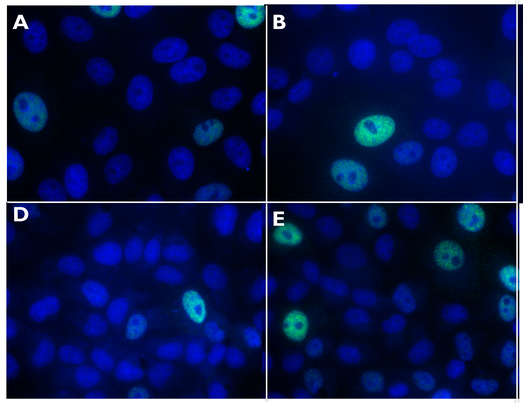

Figure 1: examples of the differences in each concentration of ursolic acid.
A: a cover slip untreated with ursolic acid.
B: treated with 5μM of ursolic acid.
C: treated with 20μM of ursolic acid.
D: treated with 50μM of ursolic acid.
E: treated with 60μM of ursolic acid.

Percentage of Infectivity

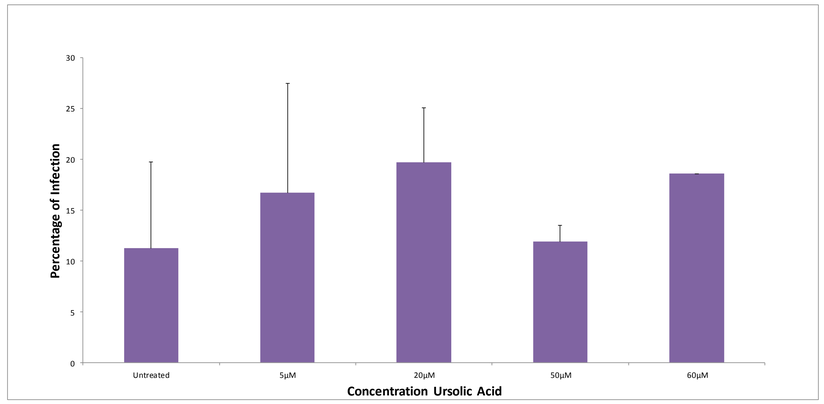

Figure 2: the percent of cells infected with the SV40 virus after being treated with various concentrations of ursolic acid. Treatment: (see Materials and Methods.) Details of the standard deviation are included. The difference in the infectivity rate was insignificant according to the p-value (.72).

Results

Analysis using one-way ANOVA indicated ursolic acid had no significant effect on infectivity of SV40 in Vero cells ($p=0.72$). When comparing the infected percentages of different concentrations, it is shown that there is no significant difference between any of them. The percentages never fall below 10 percent, and never rise above 20 percent, making them insignificant.

Discussion and Conclusion

According to the results, ursolic acid had no effect on the infectivity of the SV40 virus. Many different attributes could be a factor in this, one of them being that the Vero cells might not be affected by the ursolic acid. A to test this is to infect other types of cells with SV40, treat them with ursolic acid, and compare the infectivity rate to that of the Vero cells.

Analyzing the effects of ursolic acid on lipid-rafts would be a change to this project, so that scientists may be able to determine how much of a change ursolic acid has specifically on lipid-rafts.

In conclusion, ursolic acid does not have a significant effect on the infectivity of the SV40 virus.

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