# ANTIPROLIFERATIVE EFFECTS OF HMG-COA REDUCTASE INHIBITORS (STATINS) ARE SIGNIFICANTLY ENHANCED WHEN USED IN COMBINATION WITH γ-TOCOTRIENOL IN NEOPLASTIC MAMMARY EPITHELIAL CELLS IN CULTURE

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## Abstract

Statins, potent inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase, represent a class of antihyperlipidemic drugs. However, previous reports have demonstrated that statins also display antiproliferative and cytotoxic activity against various types of cancers. It is well established that γ-tocotrienol, a member of the vitamin E family of compounds, also displays potent anticancer activity. Studies were conducted to determine if combination therapy of simvastatin, lovastatin, mevastatin, and pravastatin with γ-tocotrienol resulted in enhanced antiproliferative effects in the highly malignant +SA mammary epithelial cells grown in culture. Cells were maintained in serum-free defined media containing EGF (10 ng/mL) and insulin (10 µg/mL) as co-mitogens. In cytotoxicity studies, cells were treated with 0-300µM of individual statins, and cell viability was assessed 24 hr later using the MTT assay. Results showed that none of the statins decrease +SA viability 24 hr after treatment exposure. In antiproliferative studies, +SA cells were treated with 0-100µM of individual statins for 4 days. Results showed that treatment with 2-8µM simvastatin, lovastatin, and mevastatin significantly inhibited +SA cell growth in a dose-responsive manner, while pravastatin had no effect on +SA cell growth at any dose tested. In order to achieve plasma concentrations of 2-8µM of these statins in humans, a treatment dose of 25 mg/kg or higher would be required, and treatment with these dose levels are associated with severe myotoxicity. However, additional studies showed that combination treatment with 0.25µM simvastatin, lovastatin or mevastatin, or 10µM pravastatin with sub-effective doses (0.25-2.0µM) of γ-tocotrienol resulted in significant dose-dependent inhibition in +SA cell proliferation during the 4 day culture period. In addition, none of the various combinations of individual statins and γ-tocotrienol were found to be cytotoxic. These findings suggest that combined treatment with γ-tocotrienol can significantly enhance the antiproliferative activity of various statins in malignant +SA mammary epithelial cells. Furthermore, the doses of statins used in these combination studies are not cytotoxic. These findings suggest that low-dose treatment of statins used in combination with low-dose γ-tocotrienol therapy may provide significant health benefits in the prevention and/or treatment of breast cancer in women, while avoiding myotoxicity associated with high dose statin treatment. Supported by NIH Grant CA 86833.

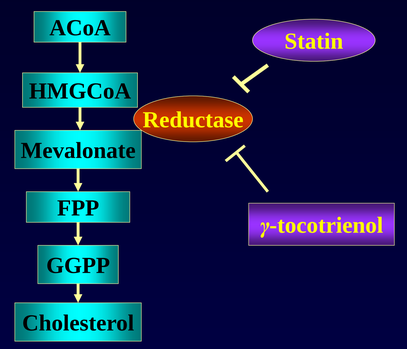
## Materials and Methods

**Cell Culture:** +SA, a highly malignant cell line was derived from an adenocarcinoma that had developed spontaneously in a female BALB/c mouse. +SA cells were maintained in serum-free defined media consisting of DMEM/F12 containing 5mg/ml BSA, 10µg/ml transferrin, 100U/ml soybean trypsin inhibitor, 100U/ml penicillin, 0.1mg/ml streptomycin, 10ng/ml EGF, and 10µg/ml insulin.

**Measurement of Viable Cell Number:** +SA cell count was determined by MTT assay. Briefly, cells were incubated at 37ºC for 4hr with media containing 0.416mg/ml MTT. Then, media was removed and MTT crystals were dissolved in 1ml isopropanol. The optical density was measured at 570nm and the number of viable cells per well was calculated against a standard curve prepared by plating various cell concentrations, as determined by hemocytometer, at the start of each experiment.

**Statistical Analysis:** Differences between various treatment groups were determined by analysis of variance followed by Dunnett's t-test. Differences were considered statistically significant at a value of $P < 0.05$.

## Mevalonate Pathway



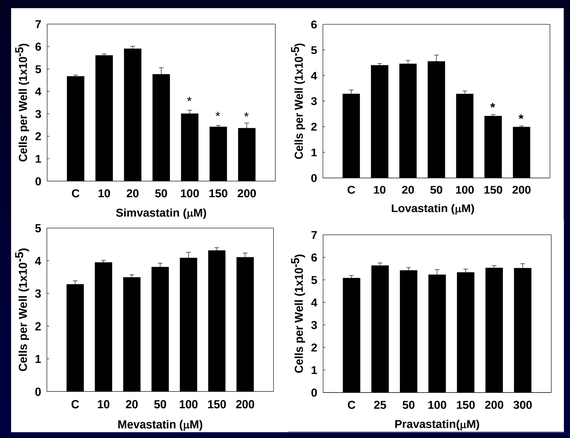




**Figure 1. Cytotoxic Study.** +SA cell viability following 24hr treatment with 0-300µM of various statins. Data points indicate mean viable cells ± SEM.

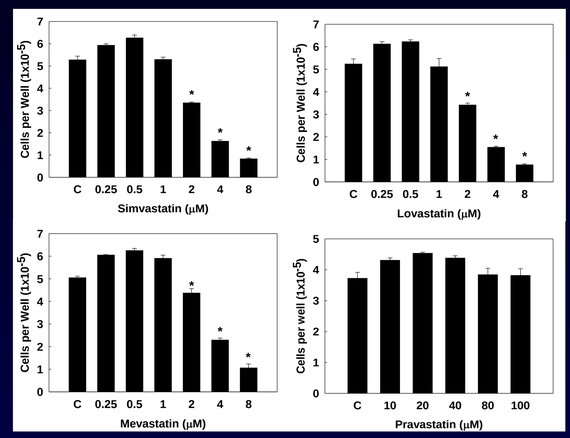



**Figure 2. Antiproliferative Study.** +SA cell growth following 4 days treatment with 0-100µM of various statins. Data points indicate mean viable cell count ± SEM.

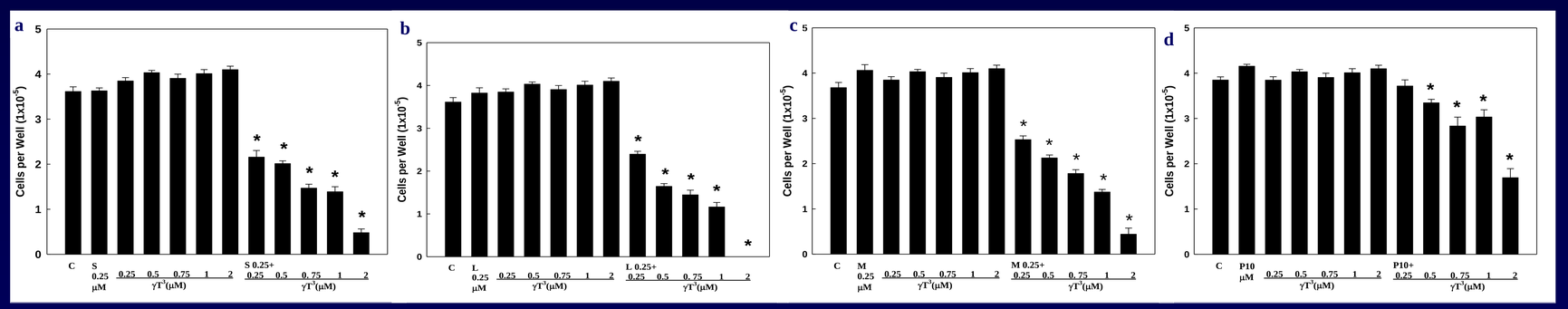
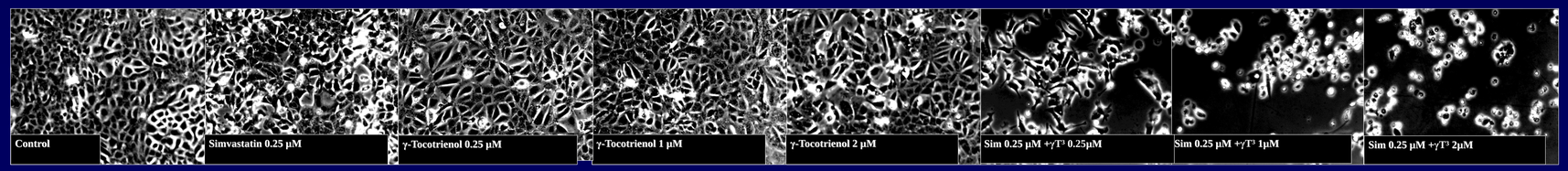



**Figure 2. Antiproliferative Study of the combination.** +SA cell growth following 4 days treatment with combination of 0.25µM of simvastatin (a), lovastatin (b) or mevastatin (c), or 10µM pravastatin (d) and 0.25-2.0µM of γ-tocotrienol. Data points indicate mean viable cell count ± SEM. Photomicrographs of +SA cells on day 5 from experiment (a) are shown below.



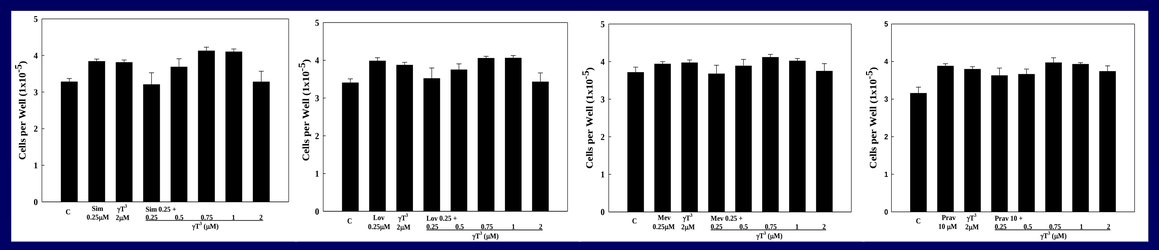




**Figure 1. Cytotoxic Study of the combination.** +SA cell viability following 24hr treatment with combination of 0.25µM of simvastatin, lovastatin or mevastatin, or 10µM pravastatin and 0.25-2.0µM of γ-tocotrienol. Data points indicate mean viable cells ± SEM.

## CONCLUSIONS

1. Statins are not acutely cytotoxic to neoplastic +SA mammary epithelial cells.
2. Simvastatin, lovastatin and mevastatin inhibited +SA cell growth in a dose-responsive manner, whereas similar doses of pravastatin had no effect on +SA cell growth.
3. Combination of subeffective doses of statins and γ-tocotrienol resulted in a significant synergistic dose-dependent inhibition of +SA cell growth.
4. Combined treatment of statins and γ-tocotrienol may provide effective anticancer therapy without the adverse side effects associated with administration of high statin doses.