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13. ABSTRACT ***(Maximum 200 Words)***

This study is examining the effects of American ginseng on human breast cancer cell proliferation *in vitro* and *in vivo*. An extract of American ginseng was shown to significantly decrease MCF-7 and MDA-MB-231 human breast cancer cell proliferation *in vitro* in a dose-dependent manner. The IC50 for ginseng extract on MCF-7 cell proliferation was 1.1 x 10^{-3} g/ml, and for MDA-MB-231 cells the IC50 was 2.3 x 10^{-3} g/ml. The ginseng extract also significantly decreased tumor size in female nude mice inoculated with MDA-MB-231 human breast cancer cells and exposed to a 1% ginseng extract in their drinking water during the experiment. Analysis of the ginseng extract using HPLC demonstrated the presence of 7 identifiable ginsenosides, Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1. When MDA-MB-231 or MCF-7 cells were incubated in the presence of a 50μM concentration of ginsenoside, only ginsenoside Rc produced profound inhibition of cell proliferation; after 8 days of treatment, Rc-exposed cells were reduced by over 75% of control. The other ginsenosides had no effect on breast cancer cell proliferation. Thus, American ginseng extract and ginsenoside Rc may have potential preventative or therapeutic effects for breast cancer in humans.

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Introduction

The ingestion of Asian ginseng or ginseng root components has been reported to reduce the risk of human cancer and has been shown to decrease the rate of occurrence and proliferation of cancers in experimental animals. However, the effects of ginseng on the prevention and treatment of breast cancer has not been studied. This research project was designed to examine the effects of American ginseng (*Panax quinquefolium*) on breast cancer by studying the effects of novel ginseng preparations 1) on well-established human breast cancer cell models *in vitro*, 2) on human mammary tumor xenografts in nude mice *in vivo*, and 3) on carcinogen-induced mammary tumor development in female rats. Our hypothesis stated that specific ginseng components would inhibit the proliferation and growth of human breast cancer cells and would reduce the incidence of mammary tumor development in experimental animals. As breast cancer is one of the most prevalent and deadly diseases afflicting women today, a means of preventing and/or treating breast cancer through dietary supplements would have a significant impact on women's health.

Body

This is an annual report of Year 1 of the grant "American Ginseng in the Prevention and Treatment of Human Breast Cancer." In the Statement of Work, two tasks were described which were to be initiated and/or finished in Year 1. In Task 1, which was testing the effects of American ginseng extract on human breast cancer tumor growth in nude mice and on mammary tumor formation in carcinogen-treated female rats, all sub-tasks have been completed. These include the preparation of ginseng extract and its HPLC analysis for ginsenoside content, treating nude mice with different concentrations of ginseng extract and inoculating them with human breast cancer cells, and treating rats with various concentrations of ginseng extract and treating them with the carcinogen NMU. The NMU study is currently underway and there is no data to report at this time. In Task 2, which was testing the effects of ginsenosides on MCF-7 and MDA-MB-231 cell proliferation *in vitro*, all sub-tasks have been initiated and will be completed in the next 6 months. This includes the treatment of MCF-7 and MDA-MB-231 cells with ginsenosides and measuring changes in cell proliferation. Much of this data will be presented below. The specific tasks as described in this grant's Statement of Work include:

Task 1. Testing effects of American ginseng extract on human breast cancer tumor growth in nude mice and on mammary tumor formation in carcinogen-treated female rats.

a. Prepare water extract of ginseng for HPLC analysis (month 1)
b. Order mice and treat water with ginseng extract (months 1-6)
c. Grow MCF-7 and MDA-MB-231 cells for inoculation (months 1-6)
d. Inoculate mice and monitor tumor growth (months 1-8)
e. Order rats and treat water with ginseng extract (months 7-12)
f. Treat rats with NMU and monitor tumor formation (months 8-18)

Task 2. Testing effects of ginsenosides on MCF-7 and MDA-MB-231 cell proliferation *in vitro*.

a. Grow and plate MCF-7 and MDA-MB-231 cells for inoculation (months 6-18)

b. Treat cells with ginsenosides and measure proliferation (months 6-18)

A water extract of ginseng was prepared as described in Methods section of grant (Task 1a). A sample of lyophilized extract was sent to the Altex Corporation for HPLC analysis of ginsenoside content. Ginsenoside standards in the analysis included ginsenoside Rb1, Rb2, Rc, Rd, Re, Rf and Rg1. As seen in Figure 1 in the Appendices, the ginseng extract contained all of the ginsenosides with identified standards in the amounts of Rb1>Rd>Re>Rc>Rg1=
Rf=Rb2. The ginsenoside standards for Rg2, Rh1, Rh2 and Ro are currently not commercially available in the United States which is why they were not included in the analysis of our extract.
These results indicate that the ginseng extract used in our *in vivo* and *in vitro* studies contain the biologically active ginsenosides which may be responsible for the effects of ginseng extract on breast cancer cells. Indeed, as shown in Figure 2, a dose-response study examining the effects of ginseng extract on MCF-7 and MDA-MB-231 indicates that ginseng extract, particularly in higher doses, inhibits cell proliferation in a dose-responsive manner. The IC_{50} of ginseng extract for MCF-7 cells was1.1 x 10^{-3} g/ml and for MDA-MB-231 cells was 2.3 x 10^{-3} g/ml. In this study, cells were plated (4 x 10^3) and treated with a specific dose of ginseng extract (≥12 wells/treatment group). Every two days (days 3 and 5 after plating on day 1), media was replaced with either plain media (control) or media containing ginseng extract (5 x 10^{-6} – 3.5 x 10^{-3} g/ml). Numbers of cells/well were counted on day 6 after plating (24 hr after last treatment).

In a second study with ginseng extract, female nude mice were given different doses of ginseng extract in their drinking water (Task 1b-d). Two weeks prior to cancer cell inoculation, mice (n=12/treatment group) were given either 0.01%, 0.1% or 1 % ginseng extract in their drinking water and treatment continued throughout the experimental period. Control mice were given water only. After two weeks of treatment, mice were inoculated with 5 x 10^6 MDA-MB-231 cancer cells in a volume of 150µl into their right flank. Tumors were measurable in cu. mm by 3 weeks after inoculation. As shown in Figure 3, mice with 1% ginseng extract in drinking water exhibited a significantly decreased tumor size at 6 weeks post-inoculation when compared to water controls. Tumor size was significantly decreased in the ginseng treated animals through week 9 of the study, at which time all animals were sacrificed due to increased morbidity in the control mice. There were no differences in tumor size between animals on the 0.01% or 0.1% ginseng extract and water controls (data not shown). These data indicate that American ginseng extract significantly decreases MDA-MB-231 cell proliferation and tumor growth. The ability of ginseng extract to inhibit cell proliferation *in vitro*, suggests that ginseng extract may directly inhibit tumor growth *in vivo* as well.

In a third study with ginseng extract (Task 1e-f) female Sprague-Dawley rats were treated with either tap water (controls) or ginseng extract (0.1% or 1%) in their tap water (n=12 rats/treatment group) for 4 weeks prior to the administration of two iv injections of N-

nitrosomethylurea (NMU; 50 mg/kg b.w.), 1 week apart, via tail vein. Animals will remain on ginseng extract throughout the experiment. Mammary tumors began to develop approximately 8 weeks following NMU treatment. As this study is on-going, final data will be presented in a later report.

In Task 2a-b, the effects of specific ginsenosides on MDA-MB-231 and MCF-7 breast cancer cell proliferation were studied. MDA-MB-231 cells were treated on the day of plating and every 2 days with 50μM of either ginsenoside Rb1, Rb2, Rc, Rd, Re or Rf (≥12 wells/treatment group). Plates of cells were counted every 2 days. As shown in Figure 4, ginsenoside Rc produced a dramatic inhibition of cancer cell by day 2 of treatment. Cell proliferation was decreased to approximately 25% of control after 8 days of treatment. Interestingly, none of the other ginsenosides affected MDA-MB-231 cell proliferation at any time after treatment. As shown in Figure 5, MCF-7 cell proliferation was similarly decreased in response to treatment with a 50μM dose of ginsenoside Rc. Dose-response experiments are currently underway to determine how a wide range of ginsenoside concentrations affects MCF-7 and MDA-MB-231 cell proliferation.

Key Research Accomplishments

- American ginseng extract inhibited MCF-7 and MDA-MB-231 human breast cancer cell proliferation *in vitro* in a dose-dependent manner.
- A 1% American ginseng extract in drinking water decreased tumor size *in vivo* in female nude mice inoculated with MDA-MB-231 human breast cancer cells.
- American ginseng extract contains 7 identifiable ginsenosides, as determined by HPLC, which may be responsible for the anti-proliferating effect of the ginseng extract on human breast cancer cells *in vitro* and *in vivo*.
- Ginsenoside Rc dramatically decreased MCF-7 and MDA-MB-231 human breast cancer cell proliferation *in vitro*. All other ginsenosides, Rb1, Rb2, Rd, Re and Rf, were without effect.

Reportable Outcomes

Abstract:

Rice, J.A., Compardo, M.T., Scolari, K.A., and Murphy, L.L. Ginsenoside Rc inhibits human breast cancer cell proliferation *in vitro*. *(Submitted to American Society for Cell Biology Annual Meeting, December 2000)*

Presentation:

Murphy, L.L. American ginseng and its effects on human breast and prostate cancers. *(Invited Oral Presentation at the International Ginseng Conference in Leeds, NY on Sept.8, 2000)*

Manuscript:

Rice, J.A., Compardo, M.T., Scolari, K.A., and Murphy, L.L. American ginseng extract inhibits human breast cancer cell proliferation *in vivo* and *in vitro. (In preparation for submission to the Journal of Experimental Biology and Medicine, 2000)*

Conclusions

The most notable findings of this research project include our results demonstrating that American ginseng extract in the drinking water of female nude mice decreases human breast cancer tumor growth, and that a component of this extract, ginsenoside Rc inhibits breast cancer cell proliferation *in vitro*. These findings are the first to suggest that ginsenoside Rc may be responsible for the anti-proliferating actions of ginseng extract on human breast cancer cell proliferation, and that ginsenoside Rc itself should be further examined as a potential preventative or therapeutic agent for breast cancer. Preliminary animal studies in nude mice support a possible role for ginsenoside Rc as a potential therapeutic agent, however, on-going studies in female rats will also examine its potential prophylactic effects as well.

References

Not applicable

Appendices

Please see Figures 1-5 on the following pages.

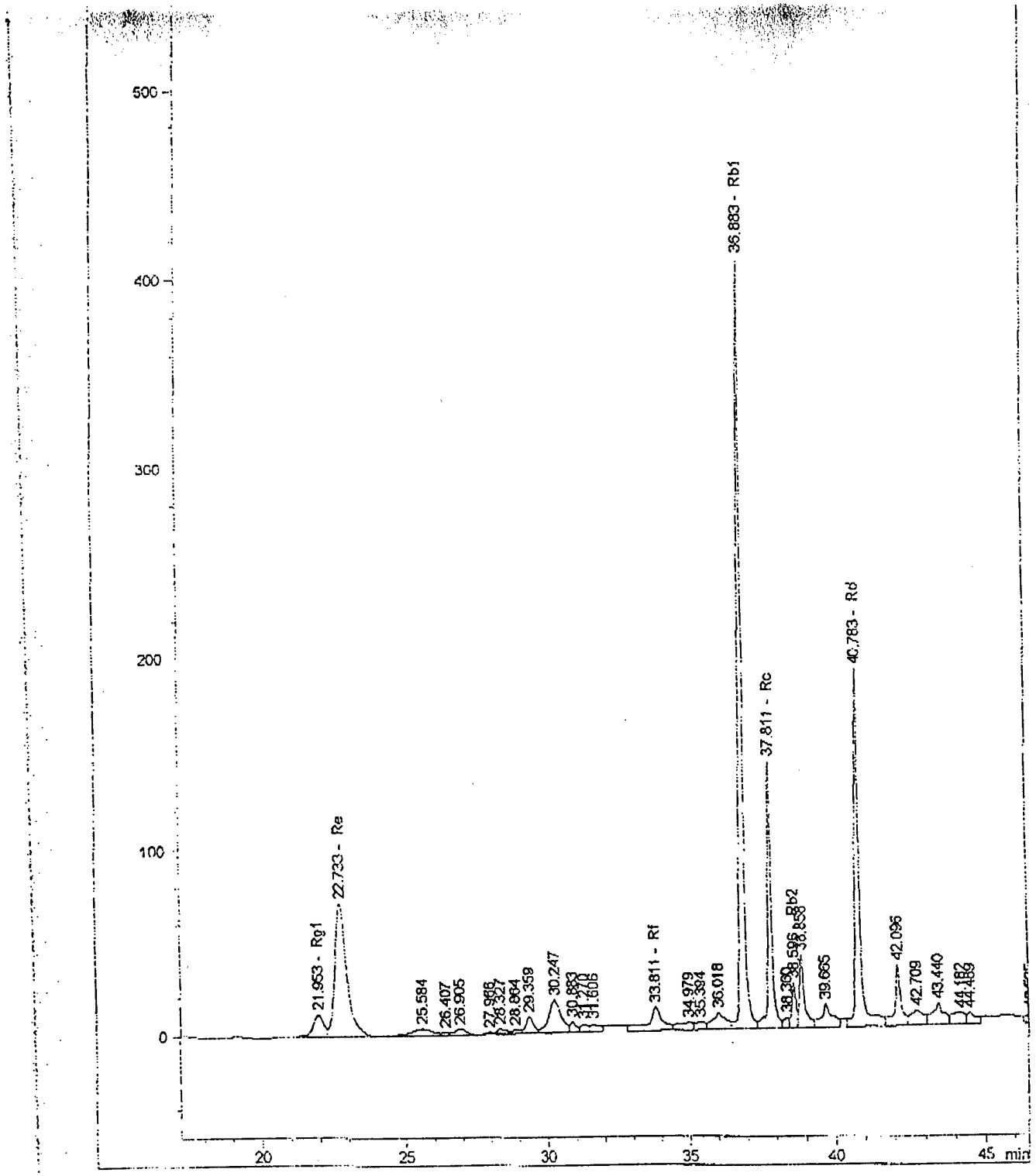

Figure 1 HPLC analysis of American ginseng extract for ginsenosides using standards for ginsenosides Rg1, Re, Rf, Rb1, Rc, Rb2, and Rd.

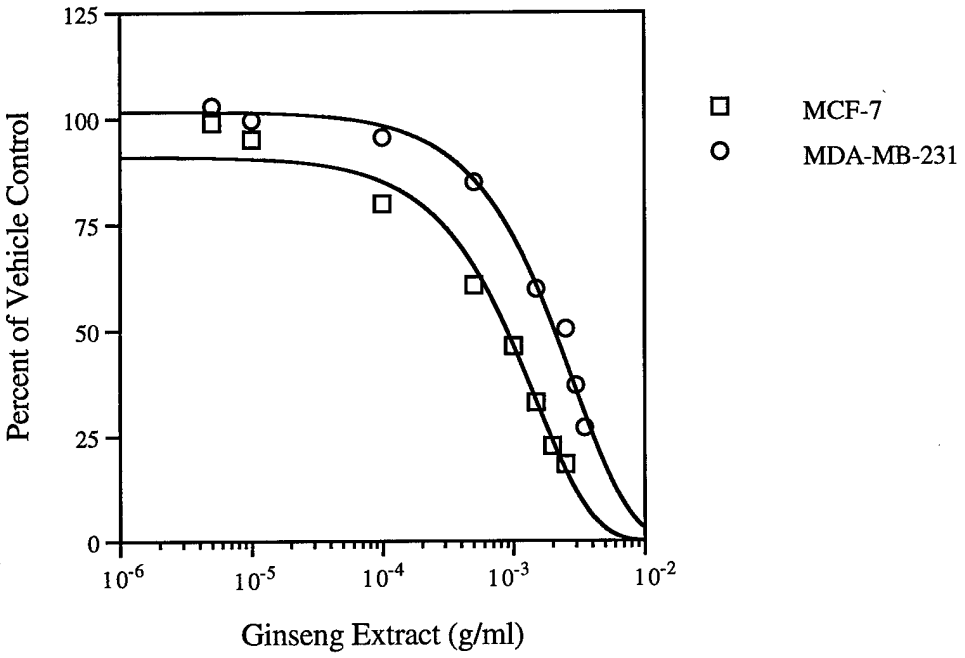

Figure 2 Effect of ginseng extract (GE) on proliferation of MCF-7 and MDA-MB-231 breast cancer cells in culture. Cells were treated with specific doses of GE every 2 days and were counted after 6 days in culture (24 hr after last treatment). Data are graphed as percent of cells relative to vehicle control.

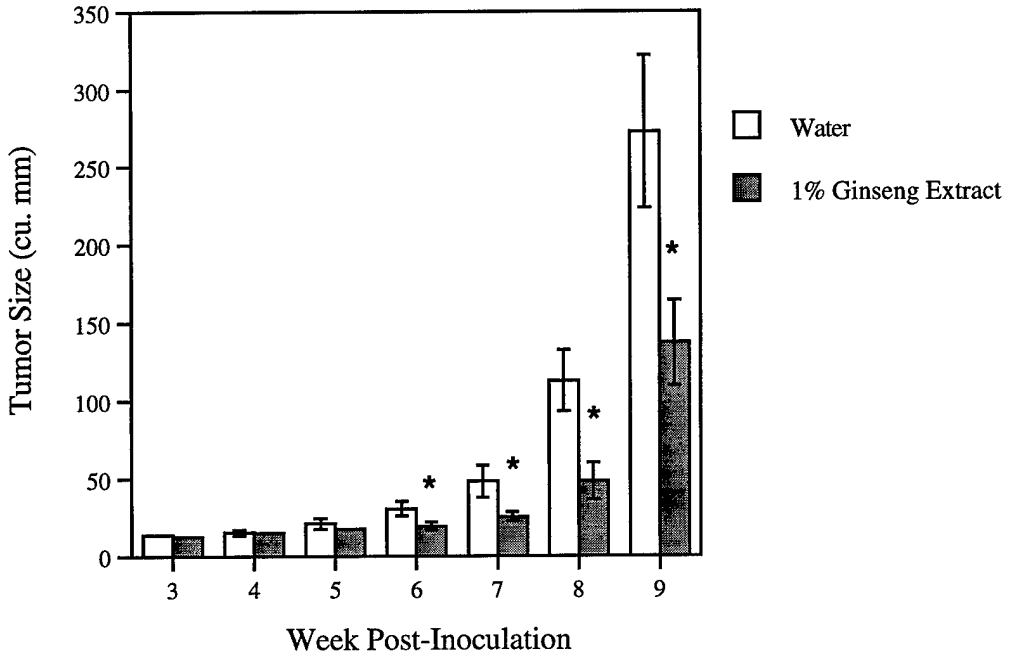

Figure 3 Effect of a 1% ginseng extract (GE) on tumor growth in female nude mice inoculated with MDA-MB-231 breast cancer cells. Drinking water containing 1% GE was provided to mice two weeks prior to inoculation with 5 x 10^6 MDA-MB-231 cells. Mice were maintained on the 1% GE throughout the experimental period. Tumor size was measured weekly. * = $p \leq 0.05$ relative to water control

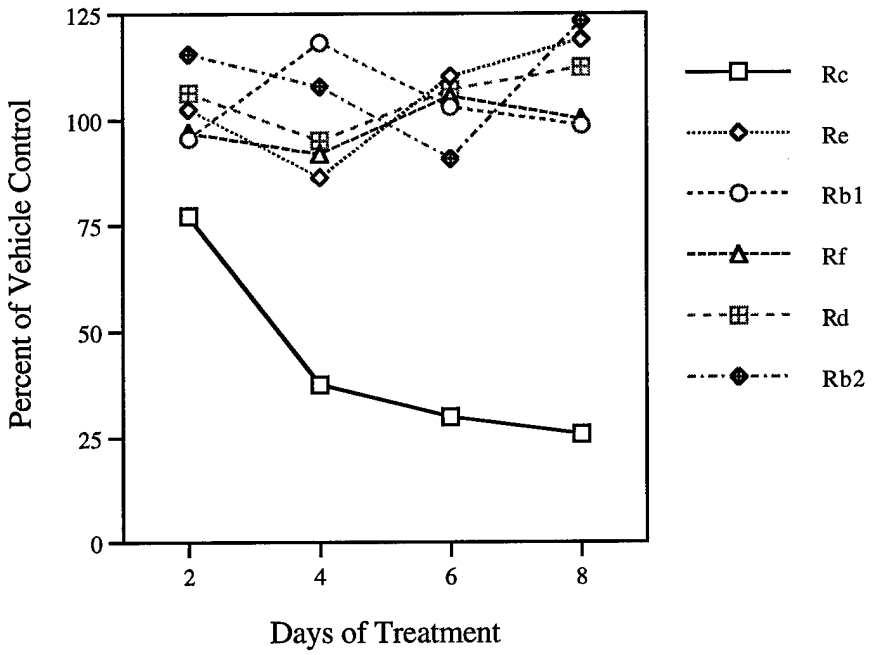

Figure 4 Effect of ginsenosides on proliferation of MDA-MB-231 breast cancer cells in culture. Cells were treated with a 50μM dose of either ginsenoside Rc, Re, Rb1, Rf, Rd or Rb2 every 2 days beginning on the day of plating (Day 0). Data are graphed as percent of cells relative to vehicle control.

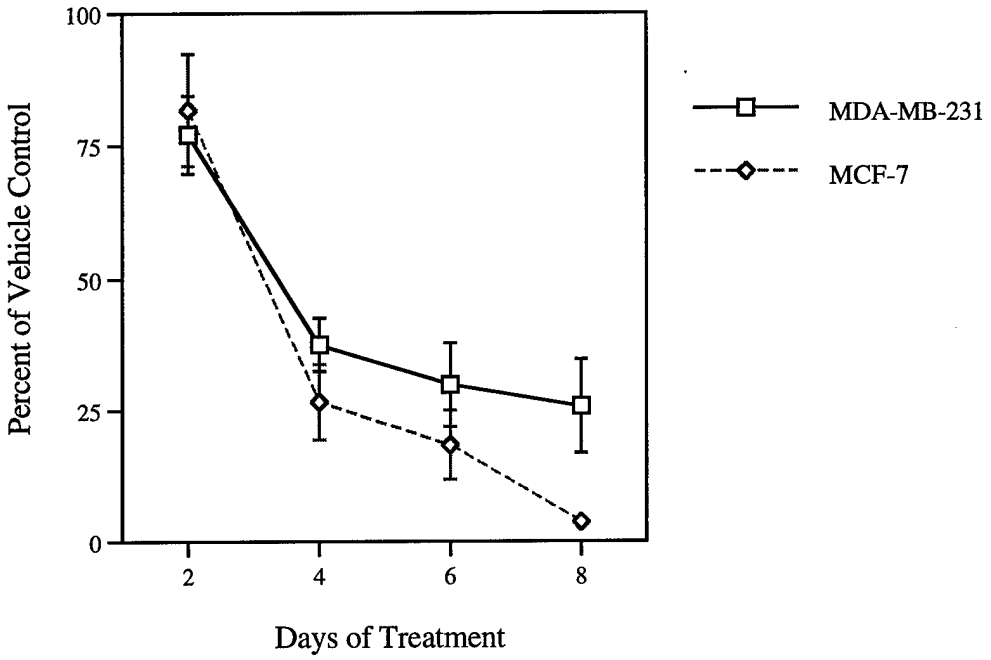

Figure 5 Effect of ginsenoside Rc on proliferation of MDA-MB-231 and MCF-7 breast cancer cells in culture. Cells were treated with a 50μM dose of ginsenoside Rc every 2 days beginning on the day of plating (Day 0). Data are graphed as percent of cells relative to vehicle control.