

## Simvastatin and tocopherol induce apoptosis in colon carcinoma cells

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**Abstract:** Apoptosis plays a significant role in tumor development and it has been hypothesized that lack/failure of apoptosis leads to the development of tumors, such as colon tumors. Thus, induction of apoptosis in tumor cells is an effective approach to the regulation of tumor growth. It has been shown that various chemo preventive agents induce apoptosis and inhibit tumor growth. Identification of agents or combinations of agents that induce tumor cell apoptosis help in the development of novel agents for colon cancer treatment. This study has been designed to assess the effectiveness of simvastatin, (a 3-hydroxy-3-methyl glutaryl-CoA reductive inhibitor), and alpha tocopherol (a micronutrient), individually or in combination on the induction of apoptosis in human HT29 colon cancer cells. HT29 cells were exposed to various doses of simvastatin and/or alpha tocopherol followed by DNA fragmentation method. Pretreatment with simvastatin (10-30 micro molar) induce apoptosis in HT29 cells at concentrations that have been studied. The same results were obtained with alpha tocopherol (5-20 micros molar). In combination, results obtained in this investigation showed significant results. In tumor cells that have been exposed to 10 micro molar simvastatin plus 5 and 10 micro molar tocopherol, cell death was not shown. According to the various mode of action and pleiotropic effects of statins in altering cell function and death, we postulate that the concentration of simvastatin is one of the most important parameter for selecting the type of action. These findings also support the hypothesis that HMGCoA-R and tocopheroles may play a role in regulation of apoptosis and provide effective strategies for the prevention of colon cancer.

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**Keywords:** Simvastatin; tocopherol; apoptosis; colon; carcinoma; cell

### 1. Introduction

Statins are HMG-CoA reductive inhibitors with important cholesterol-lowering properties. The introduction of these agents in clinical medicine has had a major impact and has changed the natural history of coronary artery disease in humans (1). Beyond their cholesterol-lowering properties, statins exhibit important anti-inflammatory and antitumor activities (1). Statins have been demonstrated to induce cell cycle arrest and cell death in various normal and cancer cells such as multiple myeloma cells (2), U266

Myeloma cells (3). Extensive studies over the last few years have demonstrated that statins generate pro-apoptotic, growth inhibitory, and pro-differentiation responses on neoplastic cells of diverse origin. In addition, several cellular pathways activated by statins have been identified and key mechanisms involved in the generation of their antitumor effects have been characterized. On the other hand alpha tocopherol, the main state of vitamin E, is a chroman cyclic from in the environment which is formed from combination of Benzene and pyran cyclic (1) Para clinic information has shown that vitamin E may send a /an Immune Anti tumor response to tumor by Macrophage and lymphocyte chemototoxicity induction(4,5). This study has been designed to assess the effectiveness of simvastatin, (a 3-hydroxy-3-methyl glutaryl-CoA reductive inhibitor), and alpha tocopherol (a

micronutrient) , individually or in combination on the induction of apoptosis in human HT29 colon cancer cells.

### 2. Material and Methods

**I- Cell culture:** HT29 cells were obtained from Pasteur institute Type Culture Collection and maintained in DMEM solution with 10% fetal bovine serum in an atmosphere of 95% O<sub>2</sub>, 5% CO<sub>2</sub> at 37°C with antibiotic.

#### II- Measurement of Cell Death by Trepan Blue Exclusion

This common cell viability assay is based on the ability of a cell with an intact membrane to exclude the dye trepan blue. The purpose of this assay was distinguished between cells with intact and disrupted membranes.

$$\% \text{ viability} = \frac{\text{number of unstained cells}}{\text{total number of cells}} \times 100$$

#### III - DNA extraction and fragmentation:

After incubation collect the cell samples in expender tube with 0.5 ml PBS and add 55ul of lysis buffer for 20 min on ice (40°C). Centrifuge at 12,000 g for 30 minutes. Then extract with phenol: chloroform (1:1) and precipitate in two equivalent of cold ethanol and one-tenth equivalent sodium acetate.

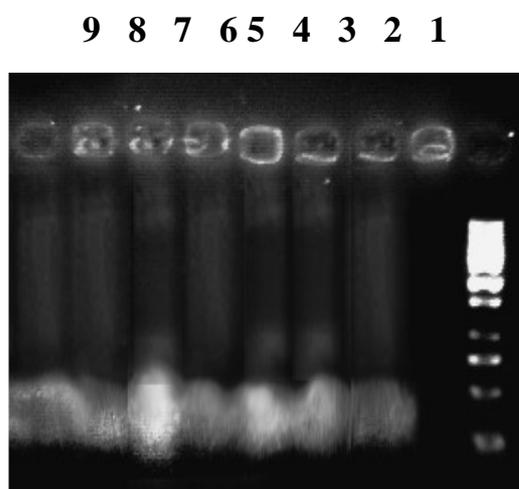
Re suspend the precipitate in 30 ul water-RNase solution and loading buffer.

#### IV- Gel electrophoresis:

Run the 1.2% gel at 5V for 5 min before increasing to 100V.

### 3. Results

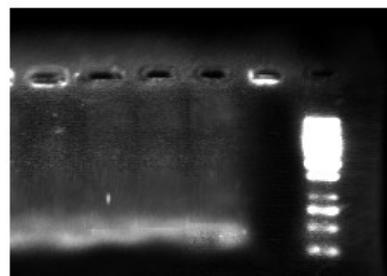
Cell viability that calculated by trepan blue was 92.3%. HT29 cells were exposed to various doses of simvastatin and/or alpha tocopherol followed by DNA fragmentation method. Pretreatment with simvastatin (10-30 micromolar) and alpha tocopherol (5-20 micro molar) induce apoptosis in HT29 cells at concentrations that have been studied (Fig 1-2).in combination results showed that simvastatin plus tocopherol induced cell death. All concentration of



**Fig 1.**The result of gel electrophoresis: HT29 cells were treated with concentrations of tocopherol( $\alpha$  T) for 14 h, then collected and handled as described in "Materials and Methods

1- 1000bp marker	4- $\alpha$ T 5 $\mu$ M
2- Negative Control	5- $\alpha$ T 10 $\mu$ M
3-Positive Control	6- $\alpha$ T 20 $\mu$ M

6 5 4 3 2 1



**Fig 2.** The result of gel electrophoresis: HT29 cells were treated with concentrations of simvastatin (SIM) for 14 h, then collected and handled as described in "Materials and Methods

1- 1000bp marker	4- SIM 5 $\mu$ M
2- Negative Control	5- SIM 10 $\mu$ M
3-Positive Control	6- SIM 20 $\mu$ M

### 4. Discussions

Para clinic information about has shown that vitamin E may send a /an Immune Anti tumor response to tumor by Macrophage and lymphocyte chemotoxity induction (4, 5). This response maybe as a result of activation of anti-tumor factors including necrotic tumor factors and p53 (6). Moreover, vitamin E and its metabolites have shown apoptotic significant effects on several cancerous human cells and in some cases have improved the effect of chemotrathy in animals. Further more, in order to increase apoptotic roads, vitamin E can control some tumor factors such as Protein kinase C .Protein kinase C is a factor which is found in several kinds of tumors. Vitamin E, especially alpha tocopherol succinate ( $\alpha$ -TS) has incredibly anti-tumor effect in prostate and breast cancers while do not induce apoptosis in normal epithelial cells (5). Induction of apoptosis cells of tumor is considered as effective methods in controlling tumor growth which is conducted in several ways. Statins are HMG-CoA reductive inhibitors with important cholesterol-lowering properties. The introduction of these agents in clinical medicine has had a major impact and has changed the natural history of coronary artery disease in humans (1). Beyond their cholesterol-lowering

properties, statins exhibit important anti-inflammatory and antitumor activities. Extensive studies over the last few years have demonstrated that statins generate pro-apoptotic, growth inhibitory, and pro-differentiation responses on neoplastic cells of diverse origin. In addition, several cellular pathways activated by statins have been identified and key mechanisms involved in the generation of their antitumor effects have been characterized (7). Recent studies indicate that increased oxidative stress may be involved in statin-induced cytotoxicity in MCF-7 breast cancer cells (8) and cervical cancer cells (9) and colon carcinoma cells too (10). According to the various mode of action and pleiotropic effects of statins in altering cell function and death, we postulate that the concentration of simvastatin is one of the most important parameter for selecting the type of action. These findings also support the hypothesis that HMGCoA-R and tocopheroles may play a role in regulation of apoptosis and provide effective strategies for the prevention of colon cancer.

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