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The Effect of Statins in Combination with Chemotherapy on Colon Cancer Cell Lines

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ABSTRACT

Background and Objective: Considering the anti-cancer effects of statins on colon cancer previously reported in epidemiological studies and uncertainty about the mechanisms of action, this study was performed to investigate the effect of statins in combination with chemotherapy on colon cancer cell lines.

Methods: The present study, which is a laboratory study, was performed on HT-29 cell line as colon cancer cell line and normal HFF cell line. After cell culture, atorvastatin at doses of 15 and 30 µM, rosuvastatin at doses of 30 and 60 µM, simvastatin at doses of 10 and 20 µM and 5-fluorouracil (5-FU) at 20 µg/ml were used for interventions in this study. Interventions were performed alone and in combination. Finally, MTT test was performed to evaluate cell viability.

Findings: Mean cell viability in simvastatin (20 µM) and simvastatin (20 µM)+5-FU (20 µg/ml) on colorectal cancer cell lines was 58.46±7.58 and 55.73±15.33 (p=0.999), respectively, in rosuvastatin (30 µM) and rosuvastatin (30 µM)+5-FU (20 µg/ml) was 91.63±10.3 and 56.2±10.27 (p=0.026), respectively, and in atorvastatin (30 µM) and atorvastatin (30 µM)+5-FU (20 µg/ml) was 82.3±26.9 and 52.16±9.49 (p=0.24), respectively.

Conclusion: The evaluation of cell proliferation showed that the addition of rosuvastatin to standard chemotherapy, unlike atorvastatin and simvastatin, had an increasing effect on cytotoxicity.

Keywords: *Statins, Cancer, Colon.*

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Introduction

Among all types of cancer, colorectal cancer is the most common cancer of the gastrointestinal tract and accounts for 38% of gastrointestinal cancers. This disease is the third most common cancer in men and the fourth most common cancer in women (except skin cancer) and its prevalence is increasing compared to previous years (1). Colorectal cancer is a multifactorial disease. Inflammatory bowel disease, low physical activity, high BMI, red meat consumption, low consumption of vegetables and fruits, smoking, family history are among the risk factors for this cancer (2).

Statins (3-Hydroxy-3-methylglutaryl-coenzyme A [HMG-CoA] reductase inhibitors) are the most potent lipid-lowering agents whose toxic role is relatively known (3, 4). The main target of statins is the mevalonate pathway, which is an important metabolic pathway. Statins inhibit the synthesis of mevalonate (an early stage in cholesterol biosynthesis) and thus inhibit downstream metabolic products in this pathway (5, 6). The mevalonate pathway not only leads to the production of cholesterol but also production of isoprenoids that are vital in various cellular processes such as cell growth and differentiation (7).

Recently, the beneficial effects of statins in reducing the incidence of colon cancer and metastatic properties have attracted special attention. A large population-based cohort study showed that long-term use of statins resulted in a relative 47% reduction in the risk of colorectal cancer after adaptation of other risk factors. Statins along with 5-FU based on preoperative radiotherapy improves pathological responses to rectal cancer, while the use of statins in postoperative treatment does not improve survival outcomes (overall survival and progression-free survival of patients with stage III colorectal cancer) (8).

Considering the effective role of statins in the treatment of cancer, which has been reported in observational studies, an in vitro analysis was performed in this study to investigate the anti-proliferative effects of statins with the aim of determining the role of different statins in combination with chemotherapy on colon cancer cell lines.

Methods

The present research is an in vitro study with ethics code IR.MAZUMS.IMAMHOSPITAL.REC.1398.019, which was conducted on HT-29 colon cancer cell lines with code C466 prepared from Pasteur Institute of Iran and HFF cell lines with code C10721 prepared from Iranian National Institute of Biological Resources.

Cells were cultured in a cell culture flask and RPMI 1640 medium in an incubator at 37° C in the presence of 5% CO2 and 97% humidity. For interventions, statins (atorvastatin, rosuvastatin and simvastatin) were used in different concentrations and 5-FU was used at a dose of 20 µg/ml. Interventions were performed alone and in combination. Doses of 15 and 30 µM were used for atorvastatin, 30 and 60 µM for rosuvastatin, and 10 and 20 µM for simvastatin.

To evaluate the effect of the 12 types of intervention in this study, MTT method was used to measure cell viability and 48 hours after the intervention, the culture medium was removed and the cells were washed 3 times with PBS. MTT powder (at a concentration of 5 m/ml dissolved in PBS) was added to each well at 25 µl concentration. The plate was covered with aluminum foil and incubated for 4 hours. The supernatant was discarded and 100 µl DMSO was then added to each well. The plate was then placed on a shaker for 20

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minutes and then read with a microplate reader at 570. The optimum density of each dose was 100 times the cell viability relative to the control group. All interventions were repeated 3 times. The obtained data were analyzed by SPSS statistical software, ANOVA and post hoc tests and $p<0.05$ was considered significant.

Results

Mean and standard deviation of cell viability in different concentrations of statins and chemotherapy on HT-29 cell line are shown in Figure 1. ANOVA test did not show a significant difference between different doses of simvastatin alone and in combination with chemotherapy as well as chemotherapy alone. Tukey test also showed no significant difference between the two groups.

Regarding rosuvastatin, ANOVA test showed a significant difference between the groups ($p=0.011$) and Tukey test also showed a significant difference between rosuvastatin and rosuvastatin with chemotherapy ($p=0.026$). ANOVA test also showed a significant difference between different groups related to atorvastatin ($p=0.033$) and Tukey test did not show a significant difference between the two groups.

Mean and standard deviation of cell viability in different concentrations of statins and chemotherapy on HFF cell line are shown in Figure 2. Statistical test showed that there was no significant difference between different doses of statins alone compared to the combined interventions of statins along with chemotherapy.

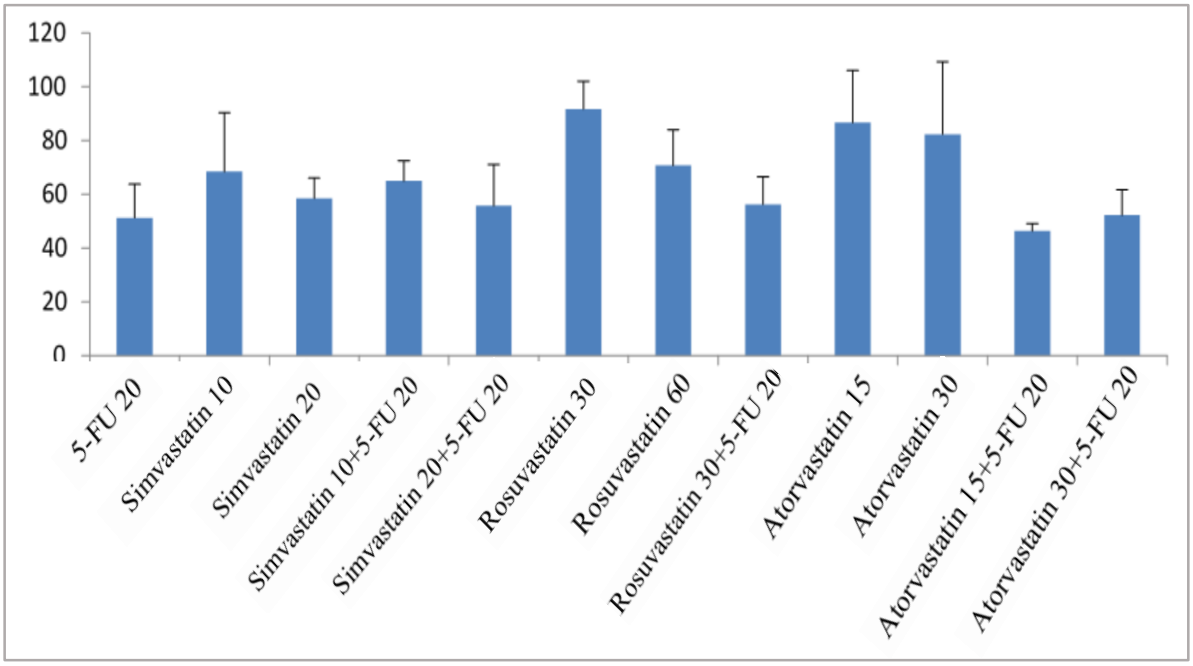

Figure 1. Comparison of mean cell viability at different concentrations of simvastatin and chemotherapy on HT-29 cell line

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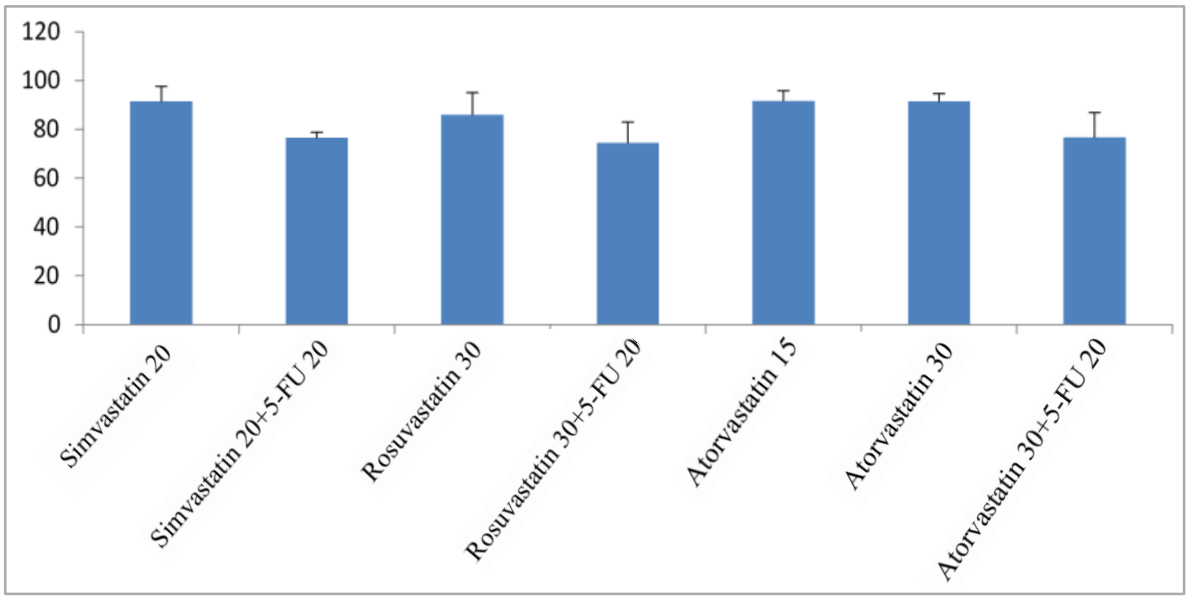

Figure 2. Comparison of mean cell viability at different concentrations of simvastatin and chemotherapy on HFF cell line

Discussion

The results of the present study showed that the addition of rosuvastatin, unlike atorvastatin and simvastatin, had an increasing effect on cell proliferation of chemotherapy medications. In most studies on cells, statins have had a significant effect on other cancer-related cellular activities. One study found the effect of lovastatin on thyroid cancer cells; Lovastatin induces pro-apoptotic activity in these cells (9).

Yang et al. showed that atorvastatin-induced activation of AMPK could induce P21 expression, which is positively associated with beclin-1 expression in CRC patients. In summary, activation of AMPK by atorvastatin increases P21 expression and ER stress response and ultimately autophagy (10). By studying cell lines for colon, ovary, breast, lung, prostate, melanoma, and brain cancers, Warita et al. showed that lowering the intracellular cholesterol levels induced by atorvastatin treatment was associated with inhibition of cancer cell growth. Exogenous expression of E-cadherin cell surface makes statin-sensitive cells relatively resistant, which means that statin resistance is partly dependent on epithelial phenotype for tumor cell uptake. Thus, just as metastatic tumor cells undergo epithelial–mesenchymal transition during the onset of metastatic cascade, statin therapy may be an effective way to target cells with high diffusion capacity (11).

The function of statins against cancer is based on inhibiting cholesterol formation and changing membrane composition, which activates proapoptotic factors such as caspase-3 and induces cell death (7, 12). In their study, Qi et al. showed that simvastatin induces the death of colon cancer cells at least in some parts by intracellular oxidative stress and induction of apoptosis (13). Comparison of the present study with other studies shows that statins play an inhibitory role on cancer by affecting the process of apoptosis and some of them, including rosuvastatin, appear to play a role in the proliferation of cancer cells. However, this discrepancy between cellular studies has also been observed in epidemiological studies of statins. In the

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study of Clancy et al., the rate of unadjusted mortality per 100,000 people per year was lower in the statin group compared to the control group. After multivariate adjustment, statin users had a lower incidence of CRC than glaucoma drugs users. In gender-disaggregated analyses, they found a protective association in men, but these results were not true for women (14). However, in a study in Finland, all statin users who had at least one prescription from 1996 to 2005 and were not diagnosed with cancer at the time of first purchase and were followed for an average of 8.8 years showed, in general, no detectable association between statins and cancer (15), or in another study in Canada, the risk of CRC among regular statin users was similar to that of people who had never used statins (16).

One of the strengths of the present study was the evaluation of the effect of statins on cell proliferation. Studies in this field are limited and one of the limitations of the present study is the lack of investigation of other mechanisms affecting the cancer process such as apoptosis.

Finally, it can be concluded that the addition of rosuvastatin, unlike atorvastatin and simvastatin along with chemotherapy drugs, has an increasing effect on the cytotoxicity of chemotherapy drugs.

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References

1.Malekzadeh R, Bishehsari F, Mahdavinia M, Ansari R. Epidemiology and molecular genetics of colorectal cancer in iran: a review. Arch Iran Med. 2009;12(2):161-9.

2.Johnson CM, Wei C, Ensor JE, Smolenski DJ, Amos CI, Levin B, et al. Meta-analyses of colorectal cancer risk factors. Cancer Causes Control. 2013;24(6):1207-22.

3.Athyros VG, Katsiki N, Tziomalos K, Gossios TD, Theocharidou E, Gkaliagkousi E, et al. Statins and cardiovascular outcomes in elderly and younger patients with coronary artery disease: a post hoc analysis of the GREACE study. Arch Med Sci. 2013;9(3):418-26.

4.Ahmadi M, Amiri S, Pecic S, Machaj F, Rosik J, Łos MJ, et al. Pleiotropic effects of statins: A focus on cancer. Biochim Biophys Acta Mol Basis Dis. 2020;1866(12):165968.

5.Corcos L, Jossic-Corcos CL. Statins: perspectives in cancer therapeutics. Dig Liver Dis. 2013;45(10):795-802.

6.Livingstone E, Hollestein LM, Herk-Sukel MP, van de Poll-Franse L, Joosse A, Schilling B, et al. Statin use and its effect on all-cause mortality of melanoma patients: a population-based Dutch cohort study. Cancer Med. 2014;3(5):1284-93.

7.Goldstein JL, Brown MS. Regulation of the mevalonate pathway. Nature. 1990;343(6257):425-30.

8.Ng K, Ogino S, Meyerhardt JA, Chan JA, Chan AT, Niedzwiecki D, et al. Relationship between statin use and colon cancer recurrence and survival: results from CALGB 89803. J Natl Cancer Inst. 2011;103(20):1540-51.

9.Di Matola T, D'Ascoli F, Luongo C, Bifulco M, Rossi G, Fenzi G, et al. Lovastatin-induced apoptosis in thyroid cells: involvement of cytochrome c and lamin B. Eur J Endocrinol. 2001;145(5):645-50.

10.Yang P-M, Liu Y-L, Lin Y-C, Shun C-T, Wu M-S, Chen C-C. Inhibition of autophagy enhances anticancer effects of atorvastatin in digestive malignancies. Cancer Res. 2010;70(19):7699-709.

11.Warita K, Warita T, Beckwitt CH, Schurdak ME, Vazquez A, Wells A, et al. Statin-induced mevalonate pathway inhibition attenuates the growth of mesenchymal-like cancer cells that lack functional E-cadherin mediated cell cohesion. Sci Rep. 2014;4:7593.

12.Hayashida K, Stahl PD, Park PW. Syndecan-1 ectodomain shedding is regulated by the small GTPase Rab5. J Biol Chem. 2008;283(51):35435-44.

13.Qi X-F, Kim D-H, Yoon Y-S, Kim S-K, Cai D-Q, Teng Y-C, et al. Involvement of oxidative stress in simvastatin-induced apoptosis of murine CT26 colon carcinoma cells. Toxicol Lett. 2010;199(3):277-87.

14.Clancy Z, Keith SW, Rabinowitz C, Ceccarelli M, Gagne JJ, Maio V. Statins and colorectal cancer risk: a longitudinal study. Cancer Causes Control. 2013;24(4):777-82.

15.Haukka J, Sankila R, Klaukka T, Lonnqvist J, Niskanen L, Tanskanen A, et al. Incidence of cancer and statin usage--record linkage study. Int J Cancer. 2010;126(1):279-84.

16.Singh H, Mahmud SM, Turner D, Xue L, Demers AA, Bernstein CN. Long-term use of statins and risk of colorectal cancer: a population-based study. Am J Gastroenterol. 2009;104(12):3015-23.

[DOR: 20.1001.1.15614107.1401.24.1.7.3]

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