行政院國家科學委員會專題研究計畫　成果報告

病理評估以 lovastatin 治療帶有人類未分化癌之裸鼠的癌細胞分化變化

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中文摘要

Lovastatin 和其他 HMG-CoA reductase inhibitors 曾被廣泛用來減少心臟血管疾病之罹病率和死亡率,但它也有其他效果.我們發現在甲狀腺未分化癌細胞株(ARO)培養, lovastatin 25 μM 能誘導細胞分化,而 50 μM 則產生細胞凋亡.同時我們發現 lovastatin 抑制 epidermal growth factor 經由 Rho/ROCK 和 FAK/paxillin signaling 的 geranylgeranylation 產生的細胞侵犯性.本實驗的目的乃觀察 lovastatin 對種上甲狀腺未分化癌之裸鼠腫瘤生長之影響. 裸鼠分成4組並觀察30天: A－陰性對照組 (n=10), 只注射 PBS; B－陽性對照組 (n=10), 有種 ARO 細胞, 注射 PBS; C－有種 ARO 細胞, 注射 lovastatin (1 mg/kg) (n=10); D－有種 ARO 細胞, 注射 lovastatin (5 m/kg) (n=10); E－有種 ARO 細胞, 注射 lovastatin (10 m/kg) (n=10).結果顯示 C 組 腫瘤生長比陽性對照組快,但 D 組第 27 天就有壓抑效果.E 組則有顯著壓抑效果(比 B 組,$P<0.01$).顯然 lovastatin 能抑制動物之甲狀腺未分化癌,值得進一步之人體臨床試驗.

關鍵辭: 甲狀腺未分化癌, 裸鼠, lovastatin

ABSTRACT

Lovastatin and other HMG-CoA reductase inhibitors have been widely used to reduce cardiovascular morbidity and mortality in many clinical studies, and they also have effects unrelated to lipid reduction. In cellular levels, we showed that treatment of human anaplastic thyroid cancer cells with lovastatin can induce cellular differentiation in a lower dose (25 μM) and cellular apoptosis in a higher dose (50 μM) (10). Later, we have revealed that lovastatin could inhibit cellular proliferation and induce apoptosis in anaplastic thyroid cancer cell lines; meanwhile, we further showed that lovastatin suppressed epidermal growth factor-induced cellular invasiveness via reduction of geranylgeranylation of Rho/ROCK and FAK/paxillin signaling. The aim of the present study was to observe the effect of lovastatin in nude mice with anaplastic thyroid cancer. The nude mice were divided into 4 groups and observed for 30 days: A—negative control group (n=10), injection of PBS only without treatment of lovastatin; B—positive control group (n=10), injection of PBS loaded with ARO cells without treatment of lovastatin; C—low dose treatment group (n=10), injection of PBS loaded with ARO cells with treatment of lovastatin (1mg/kg); D—middle dose treatment group (n=10), injection of PBS loaded with ARO cells with treatment of lovastatin (5mg/kg); E—high dose treatment group (n=10), injection of PBS loaded with ARO cells with treatment of lovastatin (10mg/kg). In group C, tumor growth seemed to be more rapid than that of positive control group, but tumor growth in group D was suppressed since Day 27. Prominent inhibition of tumor growth was shown in group E, and tumor growth volume was significantly reduced in group E in comparison with group B ($P < 0.01$). In conclusion, lovastatin can inhibit the anaplastic thyroid cancer growth in nude mice in high dose. Further human clinical trial may be warranted.

Key words: anaplastic thyroid cancer, nude mice, lovastatin

INTRODUCTION

Although only 1% of differentiated thyroid cancers transform into anaplastic thyroid cancer, this disease is invariably fatal within a few months (1). Since surgical intervention, chemotherapy and radiotherapy cannot improve the survival and life quality of such patients (2), we need to develop a brand new therapeutic approach for this fatal cancer. In recent years, we firstly found that TNFα induces the three-dimensional cytomorphological differentiation of microvilli in anaplastic cancer (3), and differentiation therapy combined radioactive iodine (^{131}I) after surgery was considered. However, TNFα might not be used to treat patients clinically for it toxicity.

Lovastatin is a 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor. Lovastatin and other HMG-CoA reductase inhibitors have been widely used to reduce cardiovascular morbidity and mortality in many clinical studies (4-6), and they also have effects unrelated to lipid reduction (7-9). In cellular levels, we showed that treatment of human anaplastic thyroid cancer cells with lovastatin can induce cellular differentiation in a lower dose (25 μM) and cellular apoptosis in a higher dose (50 μM) (10). Later, we have revealed that lovastatin could inhibit cellular proliferation and induce apoptosis in anaplastic thyroid cancer cell lines (11); meanwhile, we further showed that lovastatin suppressed epidermal growth factor-induced cellular invasiveness via reduction of geranylgeranylation of Rho/ROCK and FAK/paxillin signaling (12). Therefore, the present study tries to design an animal experiment to prove the possibility of clinical effectiveness for lovastatin in anaplastic thyroid cancer.

MATERIALS AND METHODS

HMG-CoA reductase, nude mice and cancer cell line

HMG Co-A reductase inhibitor, lovastatin, was commercially available in Taiwan. Nude mice (BALB/c-nu) were purchased from SLC Japan (Hamamatsu, Japan).

Thyroid cancer cell lines (ARO) were maintained before implantation in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml of penicillin, and 0.1 μg/ml of streptomycin at 37℃ in a humidified atmosphere of 5% CO_2. ARO cells were suspended in PBS ($4.0x10^6$ cells/ml) and implanted via direct subcutaneous injection (0.25 ml, 23G needle) over back area.

Designing of animal experiments

The nude mice were divided into 4 groups: A—negative control group (n=10), injection of PBS only without treatment of lovastatin; B—positive control group (n=10), injection of PBS loaded with ARO cells without treatment of lovastatin; C—low dose treatment group (n=10), injection of PBS loaded with ARO cells with treatment of lovastatin (1mg/kg); D—middle dose treatment group (n=10), injection of PBS loaded with ARO cells with treatment of lovastatin (5mg/kg); E—high dose treatment group (n=10), injection of PBS loaded with ARO cells with treatment of lovastatin (10mg/kg).

Tumor growth evaluation with treatment of lovastatin

Tumor growth was evaluated by its volume every three days. Lovastatin was given from the 18^{th} day in C, D, E groups with various doses. Tumor volume was calculated as: $LxS^2/2$ (L: longest length of tumor, S: shortest width of tumor) ever three days. Tumor growth volume was calculated every three days as: tumor volume detected (Day 21) minus tumor volume detected (Day 18), Day 24 minus Day 21, Day 27 minus Day 24, Day 30 minus Day 27.

Statistical Analysis

Tumor volume changes were compared using the paired Student's *t*-test, a *P* value of < 0.05 being considered statistically significant.

RESULTS

Tumor growth after implantation over nude mice was smooth and no definite tumor necrosis was noted during experimental course. Tumor growth curve of positive control group B was shown in Fig.1.

In group C (treatment with lovastatin 1 mg/kg), tumor growth seemed to be more rapid than that of positive control group (Fig. 2), but tumor growth in group D (treatment with lovastatin 5 mg/kg) was suppressed since Day 27 (Fig. 3). Prominent inhibition of tumor growth was shown in group E (treatment with lovastatin 10 mg/kg) (Fug. 4), and tumor growth volume was significantly reduced in group E in comparison with group B (positive control group), $P < 0.01$, (Fig. 5).

It is surprising and interesting to find the variation of tumor growth in various groups, because tumor growth in group C (treatment of lovastatin 1 mg/kg) is significantly faster than that in group B (positive control group). The average tumor growth rate of various groups were group E (0.0735 cm^3/3-day) < group D (0.1602 cm^3/3-day) < group B (0.2007 cm^3/3-day) < group C (0.2849 cm^3/3-day) (Table 1).

DISCUSSION

Thyroid cancer is an important endocrinological malignancy. In previous studies, one-third well-differentiated thyroid cancers may transform to poorly-differentiated patterns. Especially, 1% of well-differentiated thyroid cancer will transform to anaplastic thyroid cancer, which is fatal without effective therapeutic strategies, including surgical intervention, chemotherapy and radiotherapy.

In our prior publications, we found that lovastatin, one of HMG Co-A reductase inhibitors, can induce apoptosis in anaplastic thyroid cancer cell line with higher doses

via inhibiting Rho signaling. In thyroid tissue, cellular apoptosis to inhibit cellular proliferation play a pivotal role in maintaining homeostasis. The present study showed a strong evidence that lovastatin can inhibit human anaplastic thyroid cancer in animal models. It may point out that lovastatin could be a new therapeutic strategy in clinical entity. However, the effects of inhibiting tumor growth were noted in high dose of lovastatin (10 mg/kg), and this dose was 8 to 10 times for update human maximal dosing for hyperlipidemia. Therefore, liver dysfunction and rhabdomyolysis are the main problems in clinical trial in the future. On the other hand, it is not known why lower dose (1mg/kg) lovastatin treatment will enhance tumor growth in our experiments. We wonder that physiological dosing of lovastatin may be related with angiogenesis, but higher dose lovastatin may inhibit tumor growth, even inhibit angiogenesis.

References

1. **Goretzki PE, Simon D, Frilling A, Witte J, Reiners C, Grussendorf M, Horster FA, Roher HD.** 1993 Surgical reintervention for differentiated thyroid carcinoma. Br J Surg. 80:1009-1012.
2. **Venkatesh YS, Ordonez NG, Schulta PN, Hickey RC, Goepfert H, Samaan NA.** 1990 Anaplastic carcinoma of the thyroid: a clinicopathological study of 121 cases. Cancer. 66:321-330.
3. **Wang CY, Zhong WB, Chang TC, Lai MS, Tsai YF.** 2002 Tumor necrosis factor-α induces three-dimensional cytomorphological differentiation of human anaplastic thyroid carcinoma cells via activation of NF-κB. Cancer. 95:1827-1833.
4. **Scandinavian Simvastatin Survival Study Group.** 1994 Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet. 344:1383–1389.
5. **Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, McKillop JH, Packard CJ, West of Scotland Coronary Prevention Study Group.** 1995 Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. N Engl J Med. 333:1301–1307.
6. **Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, Brown L, Warnica JW, Arnold JM, Wun CC, Davis BR, Braunwald E, Cholesterol and Recurrent Events Trial Investigators.** 1996 The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. N Engl J Med. 335:1001–1009.
7. **Massy ZA, Keane WF, Kasiske BL.** 1996 Inhibition of the mevalonate pathway: benefits beyond cholesterol reduction? Lancet. 347:102–103.
8. **Vaughan CJ, Murphy MB, Buckley BM.** 1996 Statins do more than just lower

cholesterol. Lancet. 348:1079 –1082.

9. **Guijarro C, Egido J.** 1997 Modulation of the mevalonate pathway: potential mechanisms of vascular protection independent of cholesterol reduction. Cardiovasc Risk Factors. 7:29 –33.
10. **Wang CY, Zhong WB, Chang TC, Lai SM, Tsai YF.** 2003 Lovastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, induces apoptosis and differentiation in human anaplastic thyroid carcinoma cells. J Clin Endocrinol Metab. 88:3021-3026.
11. **Zhong WB, Wang CY, Chang TC, Lee WS.** 2003 Lovastatin induces apoptosis of anaplastic thyroid cancer cells via inhibition of protein geranylgeranylation and de novo protein synthesis. Endocrinology. 144:3852-3859.
12. **Zhong WB, Liang YC, Wang CY, Chang TC, Lee WS.** 2005 Lovastatin suppressed invasiveness of anaplastic cancer cells by inhibiting Rho geranylgeranylation and RhoA/ROCK signaling. 12:615-629.

Figure 1 Tumor size change in nude mice without treatment of lovastatin

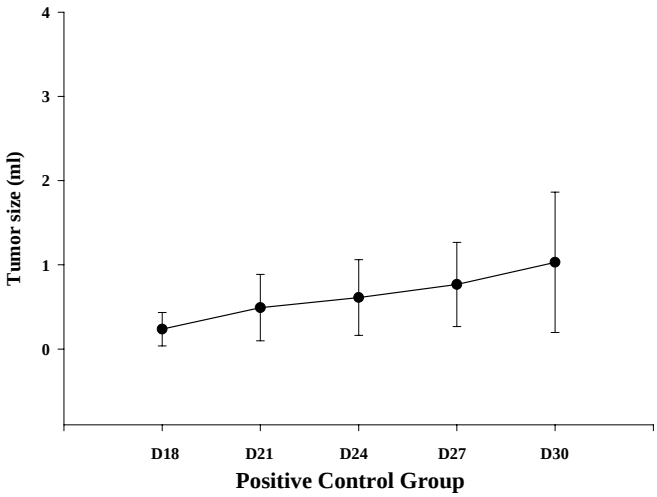

Figure 2 Tumor size change in nude mice with treatment of lovastatin (1mg/kg)

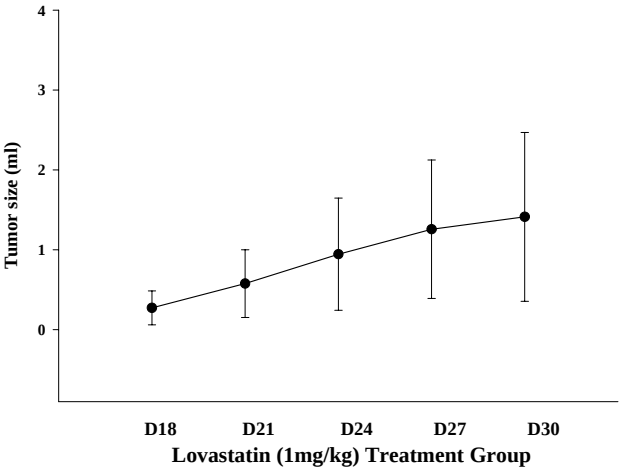

Figure 3 Tumor size change in nude mice with treatment of lovastatin (5mg/kg)

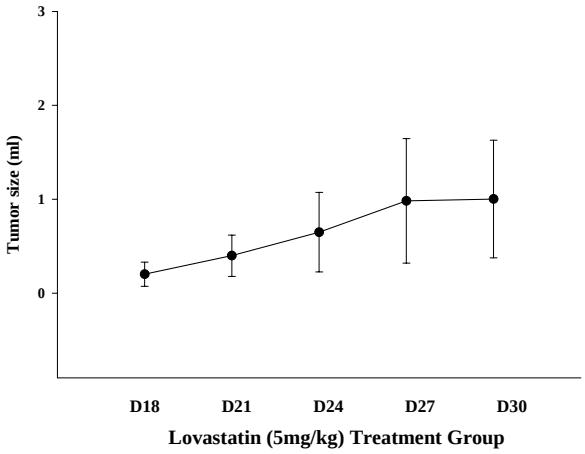

Figure 4 Tumor size change in nude mice with treatment of lovastatin (10mg/kg)

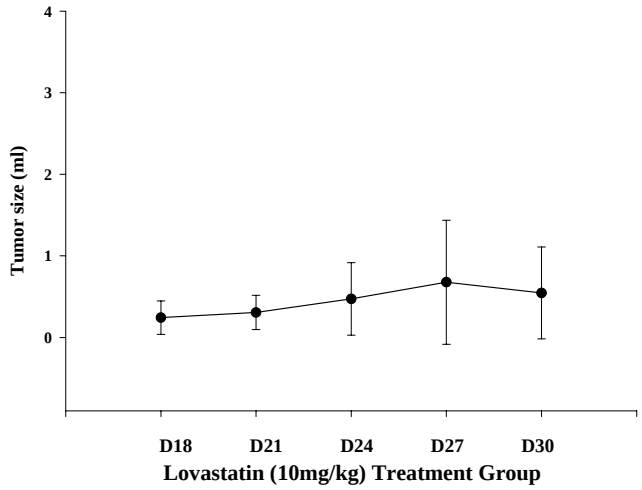

Figure 5 Tumor growth volume (Day 30 minus Day 18) in positive control group and Lovastatin (10mg/kg) group

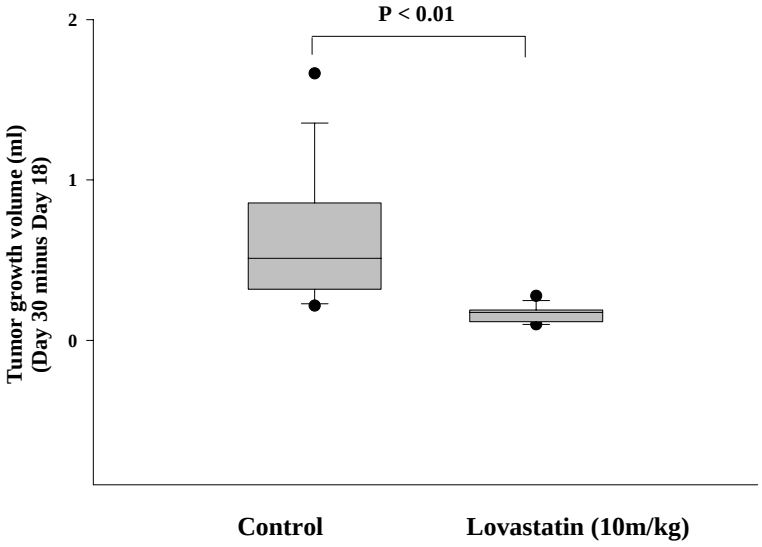

Table 1 Tumor size change (cm^3) before and after treatment of Lovastatin

	Before Treatment (Day1 to Day 18) (cm^3)	**After Treatment (Day 18 to Day 30) (cm^3)**
A	0.0000± 0.0000	0.0000± 0.0000
B	0.0395± 0.0365	0.2007± 0.1777
C	0.0453± 0.0352	0.2849± 0.2154
D	0.0273± 0.0232	0.1602± 0.1398
E	0.0407± 0.0377	0.0735± 0.1009

A: negative control; B: positive control; C: Lovastatin treatment (1mg/kg); D: Lovastatin treatment (5mg/kg); E: Lovastatin (10mg/kg)