RAPID COMMUNICATION Jpn. J. Cancer Res. 83, 1120–1123, November 1992

Effect of Pravastatin, a Potent 3-Hydroxy-3-methylglutaryl-coenzyme A Reductase Inhibitor, on Survival of AH130 Hepatoma-bearing Rats

Sumio Kawata,[1] Hiroki Kakimoto, Hiroshi Ishiguro, Eiji Yamasaki, Yoshiaki Inui and Yuji Matsuzawa

Second Department of Internal Medicine, Osaka University Medical School, 1-1-50 Fukushima, Fukushima-ku, Osaka 553

3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor is known to have an inhibitory effect on cell growth in addition to a cholesterol-lowering effect. This study examined the effect of pravastatin, a potent inhibitor of HMG-CoA reductase, on the survival of AH130 hepatoma-bearing rats. Pravastatin (1, 2, or 8 mg/kg body weight) was intraperitoneally injected once a day into tumor-bearing rats. The difference in the survival curves was significant between the controls and the rats treated with 8 mg/kg of pravastatin ($P<0.019$ by logrank test) but not between the controls and the rats treated with 1 or 2 mg/kg of the inhibitor. The tumor volume was significantly decreased in the rats treated with 8 mg/kg of pravastatin ($P<0.05$). These observations showed that intraperitoneal injection of pravastatin could improve the survival of AH130 hepatoma-bearing rats and had an inhibitory effect on the growth of the ascites form tumor.

Key words: Hepatocellular carcinoma — AH130 hepatoma — Cholesterol biosynthesis — HMG-CoA reductase

3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the major rate-limiting enzyme in cholesterol biosynthesis, has been suggested to be positively correlated with DNA synthesis and cell growth in mammalian cells.[1,2] Increased cholesterol biosynthesis has been reported in cancer cells.[3,4] In addition to serving as a precursor for the structural cholesterol requirements of cell proliferation, mevalonic acid production by HMG-CoA reductase seems to regulate cell growth, independently of cholesterogenesis, by playing a direct role in DNA synthesis.[5] Recently, cellular proteins which participate in regulation of cell growth, such as *ras* p21 and lamin A and B, have been shown to be covalently modified by mevalonate-derived farnesyl isoprenoid in the COOH-terminal.[6–8]

The mevalonate pathway, by producing isoprenoids, participates in diverse cellular functions, ranging from cholesterol synthesis to growth control through several regulatory mechanisms. It is logical to expect that inhibition of isoprenoid formation may serve as a new type of anti-cancer therapy. In addition to cholesterol-lowering activity, HMG-CoA reductase inhibitors exhibit cytostatic activity when added to proliferating cells in cell culture[9,10] and *in vivo*.[11] However, there has been no report on any survival-prolonging effect of HMG-CoA reductase inhibitor in tumor-bearing animals.

In this study, we examined the survival rate of AH130-bearing rats treated with pravastatin to test the anti-tumor effect of the HMG-CoA reductase inhibitor *in vivo*. AH130 ascites tumor was transferred intraperitoneally by inoculation of 10^7 cells into male Donryu rats weighing about 200 g (purchased from Nihon Dobutsu Co.).

Experiment 1: Survival rate in AH130-bearing rats treated with pravastatin. The AH130-bearing rats were divided into four groups: group 1 (untreated controls, n=13), group 2 (treated with pravastatin at the daily dose of 1 mg/kg body weight, n=12), group 3 (treated with pravastatin at the daily dose of 2 mg/kg, n=11), and group 4 (treated with pravastatin at the daily dose of 8 mg/kg, n=12). Pravastatin dissolved in 1 ml of saline was intraperitoneally injected once a day from the day after the tumor cells had been transplanted. The untreated control rats (group 1) were injected with saline alone. All groups of rats were housed with free access to a standard commercial chow (Oriental Yeast Co.). Water was supplied *ad libitum*. Survival was calculated from the date of the start of the treatment to the last follow-up date or death. Survival curves were constructed according to the Kaplan-Meier method.[12] The logrank test[13] was used to assess the significance of the difference in survival.

Experiment 2: Effect of pravastatin on ascites tumor mass and lipid composition in AH130-bearing rats. AH130-bearing rats were divided into three groups: group A (untreated controls, n=6), group B (treated with pravastatin at the daily dose of 2 mg/kg body weight, n=6), and group C (treated with pravastatin at

[1] To whom all correspondence should be addressed.

the daily dose of 8 mg/kg, n=6). Pravastatin dissolved in saline was intraperitoneally injected once a day from the day after the transplantation for six days, and then the rats were killed under ether anesthesia after 24 h starvation. The tumor was collected from the peritoneal cavity and its volume was determined. The numbers of tumor cells and non-tumor cells were also counted with a hematocytometer. Non-tumor cells were less than 5% of the total number of tumor cells. Lipids were extracted from plasma by the procedure of Folch *et al.*[14] and from pellets of the tumor cells by the method of Bligh and Dyer[15] as described previously.[4,16] Plasma total cholesterol was measured by gas-liquid chromatography as described previously.[16] Free cholesterol concentration in the lipid extract from the tumor cell pellets was determined using a commercial enzymatic kit (Wako Chemical Co.). Lipid phosphorus was measured as inorganic phosphorus following digestion with H_2SO_4 at 180°C as described previously.[16] Statistical analysis was carried out by using the Mann-Whitney U test.[17]

Survival curves for AH130-bearing rats treated with pravastatin (1, 2, 8 mg/kg body weight) are shown in Fig. 1. The differences between the curves of group 1 (the controls) and groups 2 and 3 (the rats treated with pravastatin at 1 and 2 mg/kg body weight, respectively) were not significant (P=0.674 and P=0.238). However, the difference between the curves of group 1 and group 4 (the rats treated with 8 mg/kg body weight) was signifi-

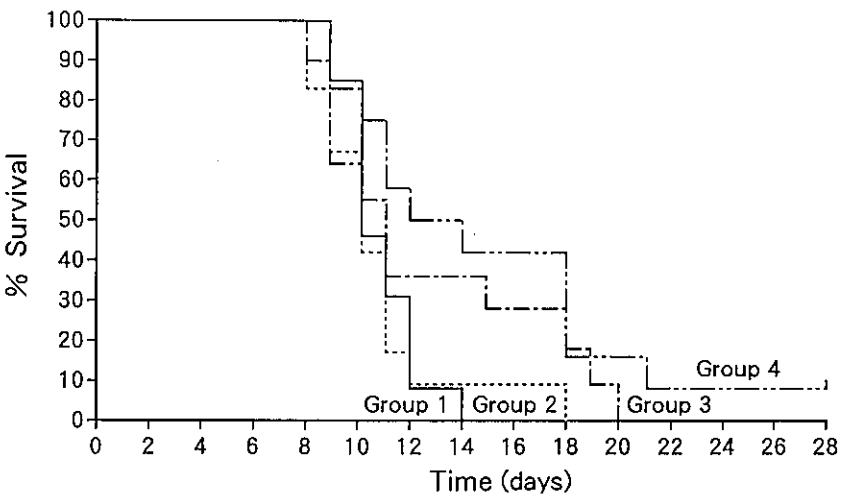

Fig. 1. Survival curves for AH130-bearing rats treated with pravastatin. Group 1, controls; group 2, rats treated with intraperitoneal injection of pravastatin at 1 mg/kg body weight once a day; group 3, rats treated with intraperitoneal injection of pravastatin at 2 mg/kg body weight once a day; group 4, rats treated with intraperitoneal injection of pravastatin at 8 mg/kg body weight once a day. The difference between the curves for group 1 and group 4 was significant by the logrank test (P=0.019), but the differences between the curves for group 1 and group 2 and for group 1 and group 3 were not significant (P=0.674 and P=0.238, respectively).

Table I. Effect of Pravastatin on Tumor Volume in the Peritoneal Cavity of AH130 Hepatoma-bearing Rats and Cholesterol Content and Cholesterol/Phospholipid Ratio in the Tumor Cells

Group	Tumor volume (cm^3)	Cholesterol content (μg/mg protein)	Cholesterol/phospholipid (%)
A (n=6)	7.0±1.7[a]	11.0±2.5	12.6±0.4
B (n=6)	5.2±1.0	9.6±2.4	11.5±0.6
C (n=6)	4.6±0.9[b]	7.6±2.0[b]	10.8±0.7[b]

Group A, controls; group B, rats treated with intraperitoneal injection of paravastatin of 2 mg/kg body weight once a day; group C, rats treated with 8 mg/kg body weight. The statistical analysis was carried out by use of the Mann-Whitney U test.

[a] Mean±S D.

[b] $P<0.05$ compared to group A.

cant ($P=0.019$). These results indicated that a relatively high dose of pravastatin could improve survival of the AH130-bearing rats.

Since a relatively high dose of pravastatin led to better survival, we examined whether the same dose of pravastatin (8 mg/kg body weight) had an effect on tumor growth. As Table I shows, the AH130 hepatoma volume was not significantly different between groups A and B, but was between groups A and C ($P<0.05$). This indicated that a relative high dose of pravastatin has an anti-proliferative effect on AH130 and can also improve the survival of tumor-bearing rats.

Plasma total cholesterol concentrations were not significantly different between group A (the controls) and groups B and C (the rats treated with 2 and 8 mg/kg body weight, respectively); HMG-CoA reductase inhibitors are known not to decrease plasma total cholesterol concentration in rats.[18] The free cholesterol content in the tumor cells did not differ significantly between groups A and B, but did between groups A and C ($P<0.05$) (Table I). The free cholesterol/phospholipid ratio in the tumor cells did not differ significantly between groups A and B, but did between groups A and C ($P<0.05$), suggesting cholesterol depletion in the tumor cells from the rats treated with a relatively high dose of pravastatin. This cholesterol depletion in the tumor cells seems to arise partly from inhibition of cholesterol biosynthesis in the cells.

In this study, we showed that intraperitoneal injection of pravastatin, a potent HMG-CoA reductase inhibitor, has an anti-proliferative effect on AH130 hepatoma and can improve survival in rats bearing the ascites form tumor. HMG-CoA reductase inhibitors have an anti-proliferative effect on tumor cells in culture[9, 10] and *in vivo*.[11] However, the mechanisms whereby the inhibitors suppress cell growth remain unclear, although modulations of the mevalonate pathways including cholesterol and dolichol biosynthesis and of isoprenylation of cellular proteins, such as ras p21 and lamins A and B, have been reported.[19, 20]

Our observation that the HMG-CoA reductase inhibitor improved survival of the AH130-bearing rats suggests a new approach to cancer therapy. However, the amount of pravastatin given to the rats was large as compared with that used for treatment of patients with hypercholesterolemia. Recently, Sinensky *et al.*,[19] using a Chinese hamster ovary cell line and Hela cell line, showed that the extent of inhibition of HMG-CoA reductase required to stop completely the formation of isoprenylated ras p21 and lamin A was much greater than that required for 50% inhibition of cholesterol synthesis. Thus, a much higher dose of the inhibitor seems to be needed for an anti-tumor effect in human malignancy than that administered to decrease plasma cholesterol concentration in patients with hypercholesterolemia. Further study on the cytotoxicity of HMG-CoA reductase inhibitors at the high doses required for anti-tumor effects is needed to examine the feasibility of using these inhibitors as anti-cancer drugs.

(Received April 30, 1992/Accepted August 12, 1992)

REFERENCES

1) Chen, H. W. The activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase and the rate of sterol synthesis diminish in cultures with high cell density. *J. Cell Physiol.*, **108**, 91–97 (1981).
2) Kandutsh, A. A. and Chen, H. W. Consequences of blocked sterol synthesis in cultured cells, DNA synthesis and membrane composition. *J. Biol. Chem.*, **252**, 409–415 (1977).
3) Siperstein, M. D. and Fagan, V. M. Deletion of the cholesterol-negative feedback system in liver tumor. *Cancer Res.*, **24**, 1108–1115 (1964).
4) Kawata, S., Takaishi, K., Nagase, T., Ito, N., Matsuda, Y., Tamura, S., Matsuzawa, Y. and Tarui, S. Increase in the active form of 3-hydroxy-3-methylglutaryl coenzyme A reductase in human hepatocellular carcinoma: possible mechanism for alteration of cholesterol biosynthesis. *Cancer Res.*, **50**, 3270–3273 (1990).
5) Quesney-Huneeus, V., Wiley, M. H. and Siperstein, M. D. Essential role for mevalonate synthesis in DNA replication. *Proc. Natl. Acad. Sci. USA*, **76**, 5056–5060 (1979).
6) Goldstein, J. L. and Brown, M. S. Regulation of the mevalonate pathway. *Nature*, **343**, 425–430 (1990).
7) Hancock, J. F., Magee, A. I., Childs, J. E. and Marshall, C. J. All ras proteins are polyisoprenylated but only some are palmitoylated. *Cell*, **57**, 1167–1177 (1989).
8) Wolda, S. L. and Glomset, J. A. Evidence for modification of lamin B by a product of mevalonic acid. *J. Biol. Chem.*, **263**, 5997–6000 (1988).
9) Goldstein, J. L., Helgeson, J. A. S. and Brown, M. S. Inhibition of cholesterol synthesis with compactin renders growth of cultured cells dependent on the low density lipoprotein receptors. *J. Biol. Chem.*, **254**, 5403–5409 (1979).
10) Habenicht, A. J. R., Glomset, J. A. and Ross, R. Relation of cholesterol and mevalonic acid to the cell cycle in smooth muscle and Swiss 3T3 cells stimulated to divide by platelet-derived growth factor. *J. Biol. Chem.*, **255**, 5134–5140 (1980).
11) Maltese, W. A., Defendini, R., Green, R. A., Sheridan, K. M. and Donley, D. K. Suppression of murine neuroblastoma cells by mevinolin, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. *J. Clin. Invest.*, **76**, 1748–1754 (1985).

12) Kaplan, E. L. and Meier, P. Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.*, **53**, 457–481 (1958).
13) Peto, R., Pike, M. C., Armitage, P., Breslow, N. E., Cox, D. R., Howard, S. V., Mantel, N., McPherson, K., Peto, J. and Smith, P. G. Design and analysis of randomized clinical trials requiring prolonged observation of each patient: II. Analysis and examples. *Br. J. Cancer*, **35**, 1–39 (1977).
14) Folch, J., Lees, M. and Sloane-Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**, 497–503 (1957).
15) Bligh, E. G. and Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.*, **37**, 911–917 (1959).
16) Kawata, S., Chitranukroh, A., Owen, J. S. and McIntyre, N. Membrane lipid changes in erythrocytes, liver and kidney in acute and chronic experimental liver disease in rats. *Biochim. Biophys. Acta*, **896**, 26–34 (1987).
17) Siegel, S. The Mann-Whitney U test. *In* "Nonparametric Statistics for the Behavioral Sciences," pp. 267–294 (1956). McGraw-Hill Inc., New York.
18) Tsujita, Y., Kuroda, M., Shimada, Y., Tanzawa, K., Arai, M., Kaneko, I., Tanaka, M., Masuda, H., Tarui, C., Watanabe, Y. and Fujii, S. *Biochim. Biophys. Acta*, **877**, 50–60 (1986).
19) Shinensky, M., Beck, L. A., Leonard, S. and Evans, R. Differential inhibitory effects of lovastatin on protein isoprenylation and sterol synthesis. *J. Biol. Chem.*, **265**, 19937–19941 (1990).
20) DeClue, J. E., Vass, W. C., Papageorge, A. G., Lowry, D. R. and Willumsen, B. M. Inhibition of cell growth by lovastatin is independent of ras function. *Cancer Res.*, **51**, 712–717 (1991).