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In vitro antitumor activity of cerivastatin, a novel and potent HMG-CoA reductase inhibitor

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Angiogenesis, the growth of new blood vessels from a pre-existing microvascular bed, is of crucial importance for the growth, maintenance, and metastasis of solid tumors. Various anti-angiogenic factors are currently being investigated, indicating that angiogenesis may soon become a suitable target for novel antitumor therapies. However, most of the currently available potent angiostatic factors (angiostatin, endostatin) are small protein fragments and their clinical application may be associated with an unusual cost expense. In their recent report Vincent and colleagues [1] demonstrate an interesting, anti-angiogenic activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (HMG-CoA RI), cerivastatin. Inhibitors of HMG-CoA reductase (or statins) represent a newly discovered family of chemically related molecules, selected for their lipid-lowering effect. Statins are extensively used in medical practice, and large clinical trials have demonstrated that this class of lipid-lowering drugs greatly reduces cardiovascular-related morbidity and mortality in patients with and without coronary disease ([2]; for review see [3]). According to recent studies, the beneficial effect of statins may also be attributed to their favorable effects on vasculature [3].

In fact, we have demonstrated for the first time that lovastatin, another HMG-CoA RI, inhibits angiogenesis, and this effect was due to the decreased production of VEGF imposed by lovastatin in tumor cells [4]. Interestingly, the results of our study and the recent paper of Vincent et al. do not match that of Kureishi et al. [5], who show that statins promote angiogenesis. This discrepancy most likely depends of the type of the examined cells and the difference of the experimental model used. In our study, lovastatin effectively suppressed VEGF production by tumor cells harboring *ras* mutations. This resulted in the inhibition of bloodvessel formation, and finally in the retardation of tumor growth. These results suggest statin's remarkable anti-angiogenic and antitumor effects, particularly towards tumors harboring *ras* mutations.

Until recently, the beneficial antitumor effects of treatment with statins have been attributed only to their direct antiproliferative effects on tumor cells. Evidence was provided that statins induce cell cycle block in the G_1 phase, interfere with the function of the Ras oncoprotein, and induce a potent apoptotic response. Some of the statins are being tested in clinical trials as potential novel antitumor agents, as they have been widely used and have well-defined pharmacokinetics at the clinical level, displaying negligible adverse side effects. Strikingly, a large clinical trial with lovastatin (a total of 6605 patients), designed to study prevention of acute coronary events with lovastatin, demonstrates a significant reduction in the incidence of melanoma among lovastatin-treated patients [2].

The results of our previous study [4] and the paper of Vincent et al. [1] underline the feasibility of utilizing statins as anti-angiogenic agents in tumor therapy, especially if it is taken into account that they may be safely used to influence tumor bloodvessels on a daily basis at levels well below the maximum tolerated dose. Cerivastatin may be of particular interest, since it possesses superior lipid-lowering activity at doses equivalent to 1–3% of the doses of other statins (for review see [6]). Although recent reports showed that cerivastatin exerts the most potent antiproliferative activity in comparison to simvastatin, lovastatin and atorvastatin against smooth muscle and endothelial cells, no data exist yet about its direct antiproliferative activity against tumor cells. In order to further evaluate the antiproliferative effects of cerivastatin on tumor cell growth, we compared its cytostatic/cytotoxic activity against various tumor cells to that exerted by lovastatin and simvastatin.

We tested our hypothesis in a standard 3-[4,5-dimethylthiazol-2-yl]diphenyltetrazolium bromide assay (MTT) which was successfully applied in our previous studies [4]. In this study we tested cerivastatin on a panel of human and murine tumor cell lines, as shown in Table 1. The results of these experiments were plotted as dose–response curves and then subjected to median effect analysis using the CalcuSyn software (Biosoft, www.biosoft.com). Subsequently, the IC_{50} values (fraction of affected cells = 0.5) were calculated for each drug and cell line.

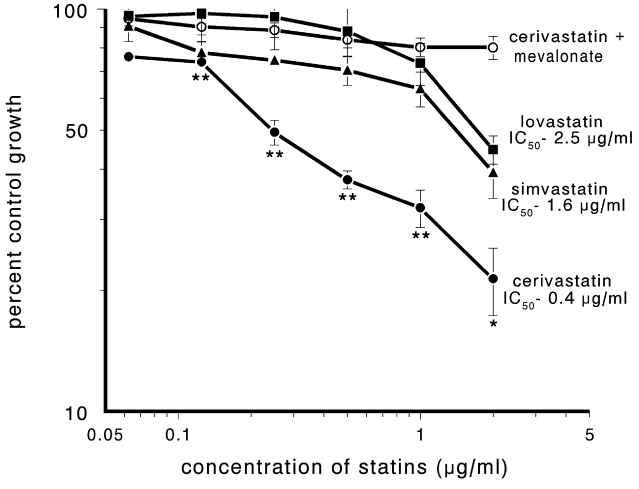

Fig. 1. Dose–response curves: effects of lovastatin, simvastatin, cerivastatin with or without mevalonic acid, on C26 colon adenocarcinoma cells in vitro. One-day-old monolayers of tumor cells were exposed to increasing concentrations (0.625–2 μg/ml) of lovastatin (■), simvastatin (▲), cerivastatin alone (●) or cerivastatin plus mevalonic acid (100 μM) (○) for 72 h. Cytostatic/cytotoxic effects, expressed as percent control growth (% of untreated control), were tested in an MTT assay. Each point represents the mean determined for quadruplicate samples (±S.D.). $*P < 0.05$; $**P < 0.001$ (Student's *t*-test) in comparison to lovastatin as reference compound.

Table 1
Inhibitory effects of lovastatin, simvastatin and cerivastatin on in vitro growth of tumor cells

Cell line	Tissue	Species	*ras* status	Cell growth assay IC_{50} (μM)[a]			
				cerivastatin	lovastatin	simvastatin	cerivastatin/lovastatin ratio
B16F10	melanoma	mouse	wild-type *ras*	1.92	5.15	3.89	0.37
C26	colon adenocarcinoma	mouse	unknown	0.84	5.94	4.01	0.14
EL-4	lymphoma	mouse	unknown	0.022	0.057	0.080	0.38
HBL-ras	Ha-*ras*-transformed mammary	human	Ha-*ras*	2.18	8.84	4.69	0.24
OVCA-1	ovary, adenocarcinoma	human	unknown	4.5	34.12	15.94	0.13
PANC-1	pancreas carcinoma	human	Ki-*ras*	9.57	138.94	122.09	0.07
Ras-3T3	Ha-*ras*-transformed fibroblasts	mouse	Ha-*ras*	0.082	4.57	1.37	0.017
T24	bladder carcinoma	human	Ha-*ras*	1.2	8.79	7.33	0.14
U937	histiocytic lymphoma	human	unknown	2.01	149.21	20.52	0.01

One-day-old monolayers of tumor cells were exposed to various concentrations of lovastatin, simvastatin or cerivastatin. Cytostatic/cytotoxic effects, expressed as percent control growth (% of untreated control), were tested in an MTT assay and subjected to median effect analysis using CalcuSyn software.

In our experiments all statins displayed a dose-dependent cytostatic/cytotoxic effect in the examined tumor cells (an example of Colon-26 tumor cells is shown in Fig. 1). However, incubation of tumor cells with cerivastatin resulted in stronger inhibition of proliferation as compared with lovastatin or simvastatin. This effect was statistically significant (Fig. 1). Moreover, the antiproliferative effect of cerivastatin on tumor cells was fully reversible by co-incubation with mevalonic acid, implying that cerivastatin's effect is the direct consequence of its ability to inhibit HMG-CoA reductase.

The antiproliferative activity of cerivastatin tested in vitro on a panel of murine and human cell lines is shown in Table 1. In this set of cell lines, cerivastatin inhibited cell growth of all lines with IC_{50} values ranging from 22 nM to 18.15 μM. Lovastatin was used as reference compound to compare the cytostatic/cytotoxic activity of cerivastatin. In vitro cerivastatin was 2.5–55 times more effective than lovastatin or simvastatin tested in the same tumor cell model. It has not been particularly effective and more specific against tumor cells harboring *ras* mutations.

Our results confirm previous observations, indicating the strong antiproliferative activity of cerivastatin [6]. In those studies cerivastatin was demonstrated to effectively inhibit proliferation of smooth muscle cells, endothelial cells and myoblasts in vitro, at $IC_{50} = 0.04–0.06$ μM [6]. In our experiments tumor cells required higher concentrations of cerivastatin than previously demonstrated in non-transformed cells. However, this effect was not identical and relied probably on the tissue source of the tumor cells.

Cerivastatin, unlike lovastatin and simvastatin, is an open ring, active form drug and belongs to the third generation HMG-CoA RI. Existing data indicate that cerivastatin is the most potent HMG-CoA reductase inhibitor amongst all reported statins. While all therapeutically used statins express their pharmacodynamic activity only in the mg range, cerivastatin's cholesterol-lowering activity is achieved in the μg range, which may offer an ultra-low dose therapy to hypercholesterolemic patients.

In conclusion, we have demonstrated a strong inhibitory effect of cerivastatin against tumor cells in extremely low doses. One may speculate that the pleiotropic effects of cerivastatin, including its potent anti-angiogenic activity, may contribute to its potential antitumor effects. These data highlight the potential of cerivastatin to include supplementation of tumor therapy. Similarly to lovastatin and simvastatin, the combination of cerivastatin with standard chemotherapeutic agents may be investigated for the potential improvement of the outcomes in the management of cancer. To our knowledge, this report is the first to demonstrate the potent cytostatic/cytotoxic effects of cerivastatin against tumor cells. However, further studies are warranted for the application of cerivastatin as a novel approach to the treatment of cancer.

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References

[1] Vincent, L., Chen, W., Hong, L., Mirshahi, F., Mishal, Z., Mirshahi-Khorassani, T., Vannier, J., Soria, J. and Soria, C. (2001) FEBS Lett. 495, 159–166.
[2] Downs, J.R., Clearfield, M., Weis, S., Whitney, E., Shapiro, D.R., Beere, P.A., Langendorfer, A., Stein, E.A., Kruyer, W. and Gotto, A.M. (1998) J. Am. Med. Assoc. 279, 1615–1622.
[3] Maron, D.J., Fazio, S. and Linton, M.F. (2000) Circulation 101, 207–213.
[4] Feleszko, W., Balkowiec, E.Z., Sieberth, E., Marczak, M., Dąbrowska, A., Giermasz, A., Czajka, A. and Jakóbisiak, M. (1999) Int. J. Cancer 81, 560–567.
[5] Kureishi, Y., Luo, Z., Shiojima, I., Bialik, A., Fulton, D., Lefer, D.J., Sessa, W.C. and Walsh, K. (2000) Nat. Med. 6, 1004–1010.
[6] Plosker, G.L., Dunn, C.I. and Figgitt, D.P. (2000) Drugs 60, 1179–1206.

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