Suppression of Murine Neuroblastoma Growth In Vivo by Mevinolin, a Competitive Inhibitor of 3-Hydroxy-3-Methylglutaryl-Coenzyme A Reductase

William A. Maltese, Richard Defendini, Robert A. Green, Kathleen M. Sheridan, and Diane K. Donley
Division of Pediatric Neurology, Department of Neurology and Division of Neuropathology, Department of Pathology, College of Physicians and Surgeons of Columbia University, and The New York State Psychiatric Institute, New York, New York 10032

Abstract

3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase catalyzes the formation of mevalonate, an essential precursor for isoprenoid compounds in mammalian cells. Recent studies have shown that mevinolin, a competitive inhibitor of the reductase, inhibits cell proliferation and induces differentiation in cultured C1300 (Neuro-2A) murine neuroblastoma cells. We now report that mevinolin can inhibit neuroblastoma growth in vivo. The specific activity of HMG-CoA reductase in subcutaneous neuroblastomas increased more than 20-fold between the fifth and eighth days after tumor inoculation, and remained elevated for the remainder of the tumor lifetime in mice. The increase in reductase activity was correlated with a marked increase in tumor DNA content and exponential increase in tumor weight. Using an in vitro assay to monitor the ability of mouse serum to suppress sterol synthesis, we determined that mevinolin was inactivated or cleared from the circulation within 3–6 h after a single subcutaneous injection. However, by using subcutaneous osmotic pumps to deliver a constant infusion of mevinolin, we were able to maintain adequate blood levels of the drug for 7 d. Mevinolin (5 mg/kg per h) suppressed tumor growth (wet weight) significantly when treatment was carried out between day 1 and day 8 or between day 5 and day 12 after tumor inoculation. Histopathological examination of tumors from mevinolin-treated mice revealed few or no mitotic figures and marked cellular degeneration. Measurements of incorporation of (^{3}H)acetate into neuroblastoma sterols and ubiquinones 24 h after implantation of osmotic pumps showed that mevinolin produced a marked inhibition of isoprenoid synthesis in the tumors in vivo. The data suggest that, in addition to their demonstrated utility as cholesterol-lowering drugs, competitive inhibitors of HMG-CoA reductase may have considerable potential as novel antineoplastic agents.

Introduction

Mevalonate is an essential precursor of various cellular isoprenoid compounds including cholesterol (1), dolichol (2, 3), ubiquinone (coenzyme Q) (4, 5), isopentenyl-transfer RNA (tRNA) (6, 7), and recently discovered isoprenoid proteins (8). The formation of mevalonate, which is catalyzed by 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA)[1] reductase, has long been regarded as the major regulatory step for *de novo* cholesterol biosynthesis (9–11). For this reason, competitive inhibitors of HMG-CoA reductase, such as compactin (ML 236B) (12) and mevinolin (MK803) (13), have received much attention as potential new therapeutic agents for treatment of hypercholesterolemia (14–21). In addition to their cholesterol-lowering activity in vivo, these inhibitors of mevalonate synthesis exhibit cytostatic activity when added to proliferating cells in tissue culture (22–27). Recent studies in this laboratory have shown that mevinolin also induces morphological and biochemical differentiation in cultured murine neuroblastoma cells (27). These observations, together with the fact that many malignant tissues exhibit relatively high activities of HMG-CoA reductase (28–33), suggested that selective inhibition of this enzyme might be exploited as a novel approach to cancer chemotherapy. Using the C1300 (Neuro-2A) murine neuroblastoma as a model system, we have shown that mevinolin effectively suppresses tumor growth in vivo.

Methods

Materials. [1-^{14}C]Acetic acid, sodium salt (57 mCi/mmol) was purchased from Amersham Corp., Arlington Heights, IL, and [^{3}H]acetic acid, sodium salt (100 mCi/mmol) was obtained from New England Nuclear, Boston, MA. Tissue culture medium and fetal calf serum were obtained from Gibco Laboratories, Grand Island, NY. Osmotic pumps for constant-infusion drug delivery (Model 2001) were purchased from Alza Corp., Palo Alto, CA. All other chemicals were from Sigma Chemical Co., St. Louis, MO.

Tumor inoculations. C1300 (Neuro-2A) murine neuroblastoma cells were obtained from the American Type Culture Collection (Rockville, MD) and maintained in monolayer culture as described previously (27). For inoculation of tumors, confluent cells were harvested by 5-min exposure to 0.25% trypsin in phosphate-buffered saline (PBS). Trypsin was inactivated by suspending the cells in Dulbecco's modified Eagle's medium containing 10% vol/vol fetal calf serum. The cells were pelleted by centrifugation and washed two times with serum-free medium. Cell number was determined with a Coulter counter (Coulter Electronics, Inc., Hialeah, FL), and the final cell suspension was diluted to 2×10^7 cells/ml. Tumors were initiated in A/J female mice (Jackson Laboratory, Bar Harbor, ME) at the age of 7 wk by subcutaneous injection of 2×10^6 neuroblastoma cells into the right ventral flank. This procedure routinely yielded 100% tumor-take. For the duration of all experiments the mice were housed individually and were allowed free access to water and standard laboratory chow.

Treatment of mice with mevinolin. Mevinolin was a gift from Alfred W. Alberts of the Merck, Sharp and Dohme Institute for Therapeutic Research, Rahway, NJ. To obtain concentrated aqueous solutions of the drug, we dissolved the lactone in absolute ethanol at 50°C to a final concentration of 27 mg/ml, and added an equal amount of solid NaOH to the ethanol solution. The ethanol was evaporated under nitrogen and

Address correspondence to Dr. Maltese, Department of Neurology, College of Physicians and Surgeons, Columbia University, 710 West 168th St., New York, NY 10032.

Received for publication 25 March 1985.

J. Clin. Invest.

0021-9738/85/11/1748/07 $1.00
Volume 76, November 1985, 1748–1754

1. *Abbreviations used in this paper:* BUN, blood urea nitrogen; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A.

the sodium salt of mevinolin was dissolved to a final concentration of 100 mg/ml in Dulbecco's PBS containing 50% (vol/vol) glycerol. The pH was adjusted to 7.5 with HCl and the solution was sterilized by passage through a 0.22-μm filter. In cases where osmotic pumps were used for constant drug infusion, animals were anesthetized with ether and the pumps were surgically implanted according to the manufacturer's instructions. All pumps were placed in a dorsal subcutaneous pocket with the outflow of the pump lying near the interscapular region, well removed from the tumor site in the flank.

Biochemical determinations. Determination of HMG-CoA reductase activity in tumor homogenates was performed by means of a radiochemical assay described previously (32). One unit of enzyme activity was defined as the formation of one picomole of mevalonate/min at 37°C.

Incorporation of radioactivity from [1-^{14}C]acetate into digitonin-precipitable sterols in cultured neuroblastoma cells was determined as described by Volpe and Hennessy (34).

Protein was determined by a microbiuret method (35), using bovine serum albumin as a standard, and DNA was quantitated by the diphenylamine reaction (36), using calf thymus DNA as the standard.

Tests for serum cholesterol and blood urea nitrogen (BUN) were performed by standard colorimetric methods, using commercially available clinical chemistry kits (Stanbio Laboratory, San Antonio, TX).

To measure the incorporation of [^{3}H]acetate into tumor lipids, the tissues were homogenized in 3.5 ml 0.15 M NaCl using a Brinkmann Polytron (Brinkmann Instruments Co., Westbury, NY), and aliquots were removed for protein determination. To 3 ml of the homogenate we added a mixture of lipid standards containing: cholesterol (60 μg), lanosterol (50 μg), cholesteryl oleate (75 μg), dolichol (15 μg), ubiquinone-10 (30 μg), squalene (10 μg), triolein (75 μg), 1,3-diolein (37 μg), 1,2-diolein (12 μg), monolein (50 μg), oleic acid (80 μg), phosphatidylcholine (10 μg), and sphingomyelin (10 μg). Lipids were extracted from the homogenates with chloroform/methanol (2:1, vol/vol), using a modification of the method of Folch et al. (37, 38). The chloroform phase was evaporated under nitrogen and the lipids were separated by two-dimensional thin-layer chromatography on Silica Gel G-25, 20 × 20 cm plates (Brinkman Instruments Co.), using solvent systems of hexane/diethyl ether/glacial acetic acid (70:30:1.5, vol/vol/vol) for the first dimension and benzene for the second dimension. The lipids were visualized by brief exposure of the plates to iodine vapor, and the zones corresponding to the lipid standards were scraped into scintillation vials and counted with 10 ml Econofluor (New England Nuclear, Boston, MA). Samples were counted in a Packard Tri-Carb liquid scintillation spectrometer (model 3255), and the counting efficiency was determined by the external standards channel ratio method. Results were expressed as disintegrations per minute incorporated into the lipid zone per gram of protein in the extracted homogenate. Alternatively, to normalize data for possible losses of material during lipid extraction, or variations in acetate uptake or pool sizes in the tumors, the incorporation of radioactivity into the sterol and ubiquinone spots on the thin-layer plates was compared with the radioactivity in the phospholipid zone, which is not decreased by mevinolin (39).

Histopathology. Tumors were resected in toto and fixed by immersion in buffered 10% formalin. After dividing the tumors into equal halves, the tissues were dehydrated, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin.

Results

To determine when the C1300 murine neuroblastoma might be most vulnerable to inhibitors of HMG-CoA reductase, we first measured the activity of the enzyme throughout the growth cycle of subcutaneous tumors. The specific activity of the reductase was low in tumors that were excised after 5 d; however, between 5 and 8 d the enzyme activity increased more than 20-fold (Fig. 1). Similar results were obtained when enzyme activity was expressed on the basis of tumor wet weight or DNA content (Fig. 1). The increase in HMG-CoA reductase activity coincided with the period of most rapid tumor growth (Fig. 2 *A*) and with a marked increase in the cellularity of the tumors, assessed by DNA content (Fig. 2 *B*).

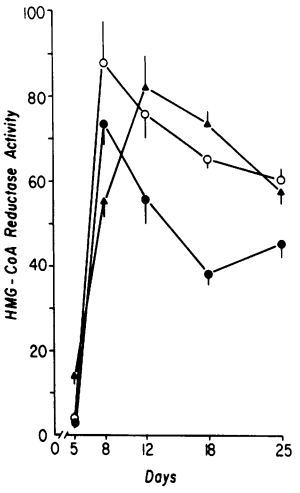

Figure 1. HMG-CoA reductase activity in murine neuroblastoma in vivo. Tumor cells were inoculated into 20 female A/J mice on day 0. On each of the indicated days, four mice were sacrificed and tumor homogenates were assayed for HMG-CoA reductase, protein, and DNA. Enzyme activities are expressed as U/mg protein (●), U/10 mg wet weight (○), and U/0.1 mg DNA (▲). The bars indicate SEM.

To establish optimal conditions for chemotherapeutic trials of mevinolin in mice, it was necessary to determine how rapidly this compound is cleared from the circulation. Groups of mice were given single subcutaneous injections of mevinolin at three different doses and blood was withdrawn from the tail vein at serial intervals after injection. By assaying the ability of 100-μl samples of mouse serum to suppress the incorporation of [1-^{14}C]acetate into sterols in cultured neuroblastoma cells, we were able to detect the presence of as little as 20 ng of mevinolin per

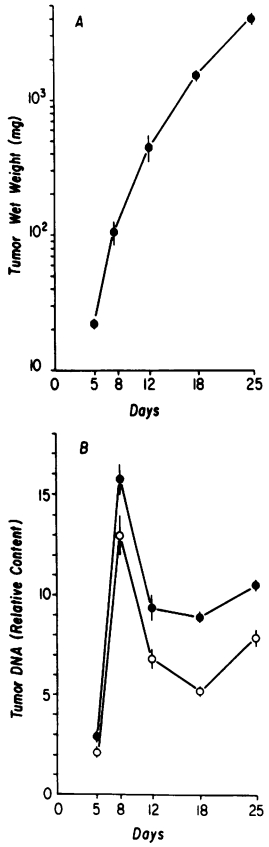

Figure 2. Growth pattern of subcutaneous neuroblastoma in mice. Tumor inoculations and biochemical determinations were performed as described in the text. The data in *A* illustrate the increase in tumor weight vs. time in the tumors used for HMG-CoA reductase assays (see Fig. 1). *B* shows the DNA content of the tumors, expressed as μg DNA/g wet weight (●), and μg DNA/0.1 mg protein (○). Each value is a mean (±SEM) derived from four tumors.

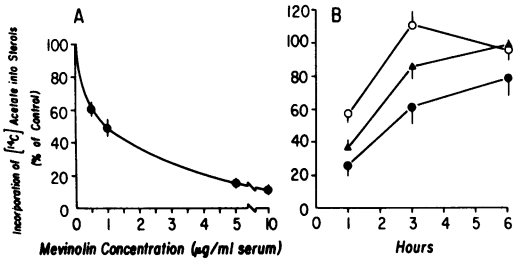

Figure 3. Duration of mevinolin activity in vivo after a single drug injection. (*A*) The in vitro bioassay developed to measure mevinolin activity in mouse blood. Neuroblastoma cells were grown in 25-cm^2 culture flasks for 24 h in Dulbecco's medium supplemented with 10% (vol/vol) lipoprotein-poor fetal calf serum. Mevinolin was mixed with mouse serum to yield the indicated final concentrations. To start the assay, each culture was incubated for 2 h at 37°C with 3 ml of fresh medium containing 100 μl mouse serum and 2 μCi [1-^{14}C]acetate. At the end of the incubation period, the cells were harvested and incorporation of radioactivity into digitonin-precipitable sterols was determined (see Methods). Each point is a mean (±SEM) of separate determinations performed on three cultures. Control cultures contained 100 μl mouse serum without mevinolin. *B* shows the results of a study in which the in vitro assay for mevinolin activity was applied to serum samples obtained from mice at the indicated time intervals after single subcutaneous injections of mevinolin at doses of 1 mg/kg (○), 5 mg/kg (▲), and 10 mg/kg (●). Control values were derived from serum samples obtained before injection of mevinolin. All blood samples were drawn from the tail vein. Each value is a mean (±SEM) derived from three mice.

ml of culture medium (0.5 μg/ml of serum) (Fig. 3 *A*). Using this bioassay, we found that most of the mevinolin derived from a single subcutaneous injection was either inactivated or removed from the circulation within 3–6 h (Fig. 3 *B*). To circumvent the problem of rapid clearance of mevinolin in mice, we resorted to the use of surgically-implanted subcutaneous osmotic pumps (Alza Corp.), which can be used to deliver a continuous drug infusion at a constant flow-rate for a period of 7 d (40).

Three experiments were carried out to assess the ability of mevinolin to suppress neuroblastoma growth in mice. In the first two trials, the pumps containing either mevinolin or vehicle solution were implanted on the day after tumor inoculation. After 7 d subcutaneous tumor growth was evident in all of the control mice, whereas tumor development in the mevinolin-treated mice was markedly stunted (Table I). In the third trial, we waited until the fifth day after tumor inoculation before commencing mevinolin treatment. Again, significant inhibition of tumor growth, based on tumor wet weight, was observed after 7 d of mevinolin treatment (12 d after tumor inoculation) (Table I).

The tumors removed from control mice and mice treated with 5 mg/kg per h mevinolin (Trial III, Table I) were examined histopathologically. The tumor histology in the four controls was the same. Tumors were densely cellular, malignant, epithelial-looking neoplasms composed of moderately large, polygonal cells with well-defined borders and no definite cell processes. Cells were arranged in sheets and the tumor was bound by a

Table I. Effects of Continuous Infusion of Mevinolin on Growth of Neuroblastoma in Mice

Treatment group	Treatment period (days after tumor inoculation)	Tumor weight	Body weight	Liver weight	Total erythrocytes	Total leukocytes	Differential WBC			BUN	Serum cholesterol	Sterol synthesis in vitro
							Lympho-cytes	Granulo-cytes	Mono-cytes			
		mg	*g*	*mg*	*10^6/mm^3*	*10^3/mm^3*	%	%	%	*mg/dl*	*mg/dl*	*% inhibition per 125 μl serum*
I. Control (n = 10)	1–8	47±23	17.2±0.6	850±30	9.7±0.2	6.9±1.4	—	—	—	—	—	0
Mevinolin (5 mg/kg per h) (n = 9)	1–8	4±1*	18.1±0.3	770±30	8.9±0.5	5.6±0.9	—	—	—	—	—	86.7±4.9
II. Control (n = 5)	1–8	116±51	18.9±0.4	—	—	—	69±8	28±7	3±1	—	—	
Mevinolin (5 mg/kg per h) (n = 3)	1–8	21±21‡	22.0±1.1	—	—	—	17±2	81±2	2±1	—	—	
III. Control (n = 4)	5–12	230±49	19.7±0.7	790±34	10.0±0.5	—	—	—	—	16.8±0.9	100±7	0
Mevinolin (5 mg/kg per h) (n = 4)	5–12	30±11§	20.7±0.8	840±45	8.6±1.5	—	—	—	—	20.8±1.1	121±2	93.9±1.8
Mevinolin (1 mg/kg per h) (n = 3)	5–12	65±13‖	19.3±0.6	910±45	8.6±0.7	—	—	—	—	15.0±1.1	123±11	74.6±2.9

* $P < 0.05$. ‡ $P < 0.10$. § $P < 0.005$. ‖ $P < 0.025$. Female A/J mice were inoculated with 2×10^6 neuroblastoma cells on day 0. We started treatment on the indicated days after tumor inoculation by implanting 7-d osmotic pumps according to the manufacturer's instructions. Pumps were filled with sterile mevinolin solution (see Methods) at a concentration designed to yield the indicated dosage, based on the average weight of the mice at the start of the experiment and assuming a constant flow rate of 1 μl/h. Control mice received pumps containing vehicle solution alone. At the end of the designated treatment period, the mice were sacrificed, tissues were removed, and the pumps were disassembled. Only mice containing pumps that had delivered their entire contents (minus the small residual volume specified by the manufacturer) were included in the study. Blood cell counts were performed with a Coulter counter. Differential counts were performed on smears stained with Wright's stain. Colorimetric determinations of cholesterol and BUN and assays for sterol synthesis-suppressing activity in mouse serum were performed as described in the text. Statistical comparison of mevinolin-treated groups to control groups were performed by Student's t test (41).

thin connective tissue capsule. Occasionally the cells formed short rows and very rarely they formed a true rosette with an acinar lumen. Most cells exhibited moderately abundant cytoplasm and an ovoid nucleus with a prominent nucleolus and one or more coarse clumps of chromatin against a pale background. Scattered tumor cells were considerably larger than the norm and had one or more large, irregular, and hyperchromatic nuclei. Near the margin of the tumor the predominant cell was smaller than elsewhere and the nuclei were more uniformly and densely chromatic. The blood supply consisted of widely spaced capillaries with modest endothelial hyperplasia and no discernible architectural relation to the tumor cells. Mitotic figures, some of them abnormal, were numerous. Spontaneous degeneration was evident in isolated cells undergoing pyknosis or simple chromatolysis and in scattered plate-like zones of coagulative necrosis.

In contrast to the control tumors, the residual neoplasms from the mevinolin-treated mice were characterized by marked cellular degeneration and depletion of mitotic cells. In the smallest of the treated tumors, there was uniform pyknotic degeneration of all the remaining tumor cells. In two other tumors, no mitotic figures were encountered and the zones of coagulative necrosis composed a larger proportion of the total area than in the control tumors. In addition, there were tissue spaces filled with proteinaceous fluid and bits of cellular debris. With the exception of the absence of mitoses, the appearance of the remaining viable tumor cells was not appreciably different from that seen in the control tumors. One treated tumor was slightly larger and better preserved than the other three. In this tumor a few cells undergoing mitosis were present. No infiltration by inflammatory cells or phagocytes was seen, and the blood vessels were not inflamed or altered. In agreement with the measurements of tumor wet weight (Table I), the surface area of the sectioned halves of the dehydrated and embedded tumors from the mevinolin-treated mice was less than one fifth that of the control tumors (Fig. 4).

Concentrated solutions of mevinolin produced some inflammatory injury, necrosis, and subcutaneous edema near the outflow site of the osmotic pumps, presumably due to the effects of high local drug levels on the dermal and epidermal tissues. However, the mevinolin-treated mice were comparable to the controls with respect to total body weight, liver weight, erythrocyte count, and BUN levels (Table I). Upon gross examination, the internal organs (e.g., heart, lungs, spleen, gastrointestinal tract) appeared normal. Total leukocyte counts also fell within the normal range; however, differential counts revealed an increased proportion of granulocytes (almost exclusively neutrophils) relative to lymphocytes in the mevinolin-treated animals. This probably reflects a stimulation of granulopoiesis in response to the local skin injury near the pump site, rather than a direct effect of mevinolin. In any case, the data suggest that mevinolin does not suppress the proliferative response of the granulopoietic and erythropoietic stem cells. Finally, we detected no obvious signs of neurotoxicity, such as ataxia, dystonia, or paralysis, in the mice treated with mevinolin.

Competitive inhibitors of HMG-CoA reductase are known to produce compensatory increases in the content of this enzyme in mouse liver (42, 43), rat hepatocytes (44), Chinese hamster ovary cells (45), and cultured human skin fibroblasts (46). We have observed a similar increase in the activity of HMG-CoA reductase in broken-cell preparations from neuroblastoma cultures exposed to mevinolin for 24 h (Maltese, W. A., and K. M. Sheridan, unpublished observation). However, as long as the competitive inhibitor is present in the culture medium, isoprenoid synthesis remains blocked in the intact neuroblastoma cells (39). Serum samples obtained from the mevinolin-treated mice at the end of the 7-d treatment regimen contained substantial sterol synthesis-suppressing activity in vitro (Table I), indicating that the osmotic pumps had delivered mevinolin for the duration of the study. However, to determine whether the level of circulating mevinolin was sufficient to block the activity of HMG-CoA reductase continuously in tumors growing in vivo, we measured the incorporation of [^{3}H]acetate into neuroblastoma sterols in mice that had been treated with a continuous infusion of mevinolin for 24 h. As shown in Table II, the level of mevinolin generated by the osmotic pumps resulted in an 80% decrease in the relative rate of sterol synthesis in the tumors. The decreased labeling of ubiquinone (Table II) indicated that

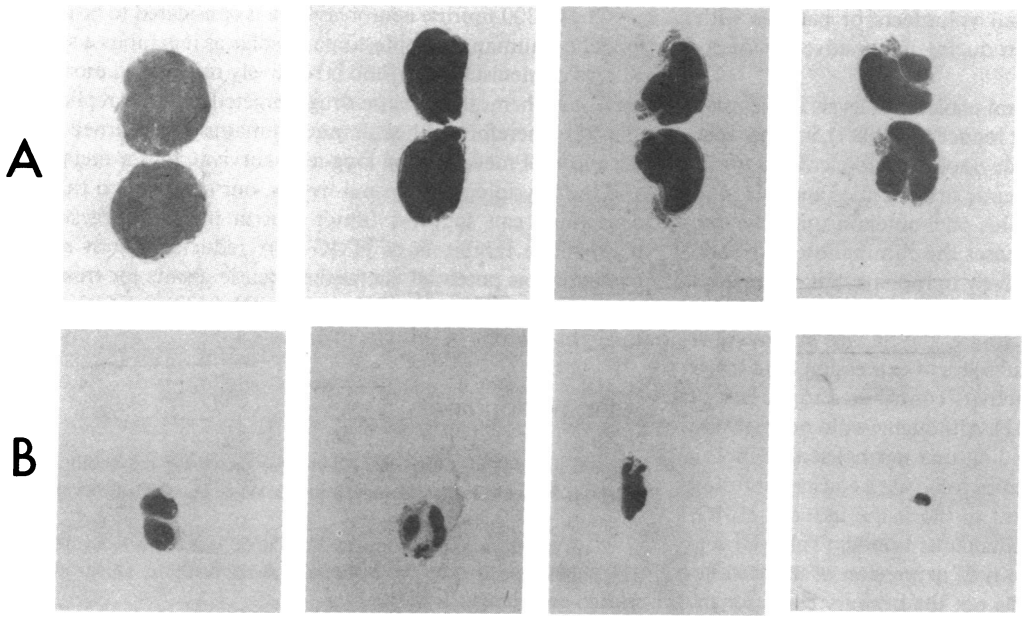

Figure 4. Photomicrographs of equatorial sections of tumors from control (*A*) and mevinolin-treated (*B*) mice. Tumors were obtained from the four control mice in treatment group III (see Table I) and from the four mice treated with mevinolin at a dose of 5 mg/kg per h in the same group. All photographs show sections stained with hematoxylin-eosin and magnified ×1.3.

Table II. Effects of Mevinolin on Incorporation of [^{3}H]Acetate into Tumor Sterols and Ubiquinones

Treatment group	Tumor weight	Sterol synthesis		Ubiquinone synthesis	
		dpm C/g protein	dpm C/dpm PL	dpm U/g protein	dpm U/dpm PL
	g				
Control (n = 3)	0.33±0.03	148,907±21,054	2.20±0.67	7,236±1,046	0.10±0.01
Mevinolin (5 mg/kg/h) (n = 3)	0.37±0.15	24,941±8.866	0.25±0.13	4,010±181	0.05±0.02

Six mice were inoculated with neuroblastoma cells on day 0 and subcutaneous tumors were allowed to develop for 10 d. On the 11th day after tumor inoculation osmotic pumps containing mevinolin or vehicle solution were implanted. 24 h later, the mice were given an intraperitoneal injection of [^{3}H]acetate (50 mCi/kg) and tumors were removed after 2 h. The incorporation of radioactivity into lipids comigrating with cholesterol (C), ubiquinone-10 (U), and phospholipids (PL) was determined as described in the *Methods*. All values are mean±SEM.

the level of circulating mevinolin was also sufficient to inhibit the flux of acetate into other branches of the isoprenoid pathway that normally seem to be saturated at low mevalonate concentrations (1).

Discussion

The present study shows that continuous infusion of mevinolin, a competitive inhibitor of HMG-CoA reductase, inhibits the growth of neuroblastoma in mice. The histopathology of the mevinolin-treated neuroblastomas suggests that the effects of mevinolin in vivo are similar to those observed in cultured cells after blocking mevalonate synthesis; i.e., initial arrest of cell proliferation (22–27) followed by eventual cell death (47). However, since foci of spontaneous degeneration were observed in the untreated tumors, it remains possible that the stunted growth of the treated tumors reflects, at least in part, attrition in the absence of new cell growth, rather than a direct cytocidal effect of the drug. The general absence of overt toxicity in mice treated with mevinolin for 7 d is consistent with the results of preliminary clinical trials in which much lower doses of this compound, administered twice daily for 1–4 wk, were found to have a cholesterol-lowering effect in normal volunteers or patients with hypercholesterolemia without producing major adverse side effects (17–20).

Our observation that the serum cholesterol levels in the mice treated with mevinolin were not reduced (Table I), is consistent with an earlier report that the mevinolin analog, compactin, is not an effective hypocholesterolemic drug in mice and rats (42). Endo et al. (42) proposed that this phenomenon might be due to the fact that compactin decreases the elimination of hepatic cholesterol via the bile acid pathway in rodents, but not in dogs or primates. However, because the animals in their study were given only one dose of compactin per day, it was also possible that rapid drug clearance and subsequent expression of induced hepatic HMG-CoA reductase activity contributed to the lack of hypocholesterolemic activity (42). Although we did not measure hepatic sterol synthesis, our finding that normal serum cholesterol levels were maintained in mice receiving a constant infusion of mevinolin at a dose sufficient to block the induced HMG-CoA reductase activity in subcutaneous tumors (Table II) suggests that the latter mechanism (i.e., expression of induced hepatic HMG-CoA reductase) was not the primary cause for the ineffectiveness of mevinolin as a cholesterol-lowering agent in our experiments. In any case, the observation that mevinolin inhibited tumor growth without decreasing serum cholesterol levels has important implications for the possible mechanism underlying the antineoplastic activity of this compound. Thus, since murine neuroblastoma cells can utilize extracellular serum low-density lipoprotein as a source of cholesterol (34, 48), it seems unlikely that inhibition of *de novo* sterol synthesis by mevinolin could by itself deprive the growing tumor cells of cholesterol needed for membrane replication. On the other hand, the data demonstrating a decreased incorporation of [^{3}H]acetate into ubiquinone in tumors from mevinolin-treated mice (Table II) are provocative, since they suggest that inhibition of neuroblastoma growth may be related to inhibition of the synthesis of nonsterol isoprenoid compounds such as dolichol, ubiquinone, isopentenyl-tRNA, or other poorly characterized isoprenoids that are required by rapidly proliferating cells. This conclusion would be consistent with studies of synchronized cells in vitro, which have shown that supplementing the cultures with cholesterol cannot reverse the arrest of cell proliferation that occurs when mevalonate synthesis is blocked by high concentrations of compactin or mevinolin (23–26).

The C1300 murine neuroblastoma is considered to be a good model for human neuroblastoma, insofar as it exhibits a similar pattern of metastasis (49) and is relatively resistant to most conventional chemotherapeutic drugs targeted at DNA replication (50–52). Therefore, although much remains to be learned about the effects of mevinolin on long-term survival, tumor metastasis, and the physiology of normal tissues, our observation that this compound can suppress tumor growth in vivo suggests that competitive inhibitors of HMG-CoA reductase merit serious evaluation as potential chemotherapeutic agents for treatment of neuroblastoma and other malignancies (32) exhibiting relatively high levels of this enzyme.

Acknowledgments

We are grateful to Alfred W. Alberts for providing mevinolin, Carol Barboni for technical assistance, and Ms. Alice H. Marti for typing the manuscript.

This investigation was supported by Public Health Service grant RO1 CA 34569, awarded by the National Cancer Institute, Department of Health and Human Services.

References

1. Brown, M. S., and J. L. Goldstein. 1980. Multivalent feedback regulation of HMG-CoA reductase, a control mechanism controlling isoprenoid synthesis and cell growth. *J. Lipid Res.* 21:505–517.

2. Gough, D. P., and F. W. Hemming. 1970. The characterization and stereochemistry of biosynthesis of dolichols in rat liver. *Biochem. J.* 118:163–166.

3. Martin, H. G., and K. J. I. Thorne. 1974. Synthesis of radioactive dolichol from (4S-^{3}H)mevalonate in the regenerating rat liver. *Biochem. J.* 138:277–280.

4. Faust, J. R., J. L. Goldstein, and M. S. Brown. 1979. Synthesis of ubiquinone and cholesterol in human fibroblasts: regulation of a branched pathway. *Arch. Biochem. Biophys.* 192:86–99.

5. Nambudiri, A. M. D., S. Ranganathan, and H. Rudney. 1980. The role of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in the regulation of ubiquinone synthesis in human fibroblasts. *J. Biol. Chem.* 255:5894–5899.

6. Hall, R. H. 1970. N^6-(Δ^2-isopentenyl)adenosine: chemical reactions, biosynthesis, metabolism, and significance to the structure and function of tRNA. *In* Progress in Nucleic Acid Research and Molecular Biology. J. N. Davidson and W. E. Cohn, editors. Academic Press, New York. 10:57–86.

7. Faust, J. R., M. S. Brown, and J. L. Goldstein. 1980. Synthesis of Δ^2-isopentenyl tRNA from mevalonate in cultured human fibroblasts. *J. Biol. Chem.* 255:6546–6548.

8. Schmidt, R. A., C. J. Schneider, and J. A. Glomset. 1984. Evidence for posttranslational incorporation of a product of mevalonic acid into Swiss 3T3 cell proteins. *J. Biol. Chem.* 259:10175–10180.

9. Rodwell, V. W., J. L. Nordstrom, and J. J. Mitschelen. 1976. Regulation of HMG-CoA reductase. *Adv. Lipid Res.* 14:1–74.

10. Goldstein, J. L., and M. S. Brown. 1977. The low-density lipoprotein pathway and its relation to atherosclerosis. *Annu. Rev. Biochem.* 46:897–930.

11. Schroepfer, G. J., Jr. 1981. Sterol biosynthesis. *Annu. Rev. Biochem.* 50:585–621.

12. Endo, A., M. Kuroda, and K. Tanzawa. 1976. Competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase by ML-236A and ML-236B, fungal metabolites having hypocholesterolemic activity. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 72:323–326.

13. Alberts, A. W., J. Chen, G. Kuron, V. Hunt, J. Huff, C. Hoffman, J. Rothrock, M. Lopez, H. Joshua, E. Harris, A. Patchett, R. Monaghan, S. Currie, E. Stapley, G. Albers-Schonberg, O. Hensens, J. Hirshfield, K. Hoogsteen, J. Liesch, and J. Springer. 1980. Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. *Proc. Natl. Acad. Sci. USA.* 77:3957–3961.

14. Yamamoto, A., H. Sudo, and A. Endo. 1980. Therapeutic effects of ML-236B in primary hypercholesterolemia. *Atherosclerosis.* 35:259–266.

15. Mabuchi, H., T. Haba, R. Tatami, S. Miyamoto, Y. Sakai, T. Wakasugi, A. Watanabe, J. Koizumi, and R. Takeda. 1981. Effects of an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase on serum lipoproteins and ubiquinone-10 levels in patients with familial hypercholesterolemia. *N. Engl. J. Med.* 305:478–482.

16. Mabuchi, H., T. Sakai, Y. Sakai, A. Yoshimura, A. Watanabe, T. Wakasugi, J. Koizumi, and R. Takeda. 1983. Reduction of serum cholesterol in heterozygous patients with familial hypercholesterolemia: additive effects of compactin and cholestyramine. *N. Engl. J. Med.* 308: 609–613.

17. Tobert, J. A., G. Hitzenberger, W. R. Kukovetz, I. B. Holmes, and K. H. Jones. 1982. Rapid and substantial lowering of human serum cholesterol by mevinolin (MK-803), an inhibitor of hydroxymethylglutaryl-coenzyme A reductase. *Atherosclerosis.* 41:61–65.

18. Tobert, J. A., G. D. Bell, J. Birtwell, I. James, W. R. Kukovetz, J. S. Pryor, A. Buntinx, I. B. Holmes, Y.-S. Chao, and J. A. Bolognese. 1982. Cholesterol-lowering effects of mevinolin, an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, in healthy volunteers. *J. Clin. Invest.* 69:913–919.

19. Bilheimer, D. W., S. Grundy, M. S. Brown, and J. L. Goldstein. 1983. Mevinolin and colestipol stimulate receptor-mediated clearance of low-density lipoprotein from plasma in familial hypercholesterolemia heterozygotes. *Proc. Natl. Acad. Sci. USA.* 80:4124–4128.

20. Illingworth, D. R., and W. E. Connor. 1984. Hypercholesterolemia persisting after distal ileal bypass: response to mevinolin. *Ann. Intern. Med.* 100:850–851.

21. Illingworth, D. R., and G. J. Sexton. 1984. Hypocholesterolemic effects of mevinolin in patients with heterozygous familial hypercholesterolemia. *J. Clin. Invest.* 74:1972–1978.

22. Goldstein, J. L., J. A. S. Helgeson, and M. S. Brown. 1979. Inhibition of cholesterol synthesis with compactin renders growth of cultured cells dependent on the low density lipoprotein receptor. *J. Biol. Chem.* 254:5403–5409.

23. Quesney-Huneeus, V., M. H. Wiley, and M. D. Siperstein. 1979. Essential role for mevalonate synthesis in DNA replication. *Proc. Natl. Acad. Sci. USA.* 76:5056–5060.

24. Habenicht, A. J. R., J. A. Glomset, and R. Ross. 1980. 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.

25. Quesney-Huneeus, V., H. A. Galick, M. D. Siperstein, S. K. Erickson, T. A. Spencer, and J. A. Nelson. 1983. The dual role of mevalonate in the cell cycle. *J. Biol. Chem.* 258:378–385.

26. Fairbanks, K. P., L. D. Witte, and D. S. Goodman. 1984. Relationship between mevalonate and mitogenesis in human fibroblasts stimulated with platelet-derived growth factor. *J. Biol. Chem.* 259:1546–1551.

27. Maltese, W. A. 1984. Induction of differentiation in murine neuroblastoma cells by mevinolin, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Biochem. Biophys. Res. Commun.* 120:454–460.

28. Siperstein, M. D., A. M. Gyde, and H. P. Morris. 1971. Loss of feedback control of hydroxymethylglutaryl coenzyme A reductase in hepatomas. *Proc. Natl. Acad. Sci. USA.* 68:315–317.

29. Kandutsch, A. A., and R. L. Hancock. 1971. Regulation of the rate of sterol synthesis and the level of β-hydroxy-β-methylglutaryl coenzyme A reductase activity in mouse liver and hepatomas. *Cancer Res.* 31:1396–1401.

30. Mitchell, A. D., T. D. Pugh, and S. Goldfarb. 1978. Partial "feedback control" of β-hydroxy-β-methylglutaryl coenzyme A reductase activity in primary hepatocellular carcinomas. *Cancer Res.* 38:4474–4477.

31. Gregg, R. G., J. R. Sabine, and P. A. Wilce. 1982. Regulation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase in rat liver and Morris hepatomas 5123C, 9618A and 5123 t.c. *Biochem. J.* 204:457–462.

32. Maltese, W. A. 1983. 3-Hydroxy-3-methylglutaryl coenzyme A reductase in human brain tumors. *Neurology.* 33:1294–1299.

33. Rao, K. N., S. Kottapally, and H. Shinozuka. 1983. Lipid composition and 3-hydroxy-3-methylglutaryl-CoA reductase activity of acinar cell carcinoma of rat pancreas. *Biochim. Biophys. Acta.* 759:74–80.

34. Volpe, J. J., and S. W. Hennessy. 1977. Cholesterol biosynthesis and 3-hydroxy-3-methylglutaryl coenzyme A reductase in cultured glial and neural cells. *Biochim. Biophys. Acta.* 486:408–420.

35. Munkres, K. O., and F. M. Richards. 1965. The purification and properties of neurospora malate dehydrogenase. *Arch. Biochem. Biophys.* 109:466–479.

36. Burton, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* 62:315–323.

37. Folch, J., M. Lees, and G. H. Sloan-Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* 226:497–509.

38. Johnson, S. M. 1978. A new specific cholesterol assay gives reduced cholesterol/phospholipid molar ratios in cell membranes. *Anal. Biochem.* 95:344–350.

39. Maltese, W. A., and J. R. Aprille. 1984. Induction of differentiation in neuroblastoma cells by mevinolin: relationship to cholesterol and ubiquinone synthesis and mitochondrial electron transport. *J. Cell Biol.* 99(4, Pt. 2):154*a* (Abstr.).

40. Theeuwes, F., and S. I. Yum. 1976. Principles of the design and operation of generic osmotic pumps for the delivery of semisolid or liquid formulations. *Ann. Biomed. Eng.* 4:343–353.

41. Brownlee, K. A. 1965. Statistical Theory and Methodology in Science and Engineering. John Wiley and Sons, Inc., New York. Second ed. 1–612.

42. Endo, A., Y. Tsujita, M. Kuroda, and K. Tanzawa. 1979. Effects of ML-236B on cholesterol metabolism in mice and rats: lack of hypocholesterolemic activity in normal animals. *Biochim. Biophys. Acta.* 575: 266–276.

43. Kita, T., M. S. Brown, and J. L. Goldstein. 1980. Feedback regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in livers of mice treated with mevinolin, a competitive inhibitor of the reductase. *J. Clin. Invest.* 66:1094–1100.

44. Edwards, P. A., S.-F. Lan, and A. M. Fogelman. 1983. Alterations in the rates of synthesis and degradation of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase produced by cholestyramine and mevinolin. *J. Biol. Chem.* 258:10219–10222.

45. Sinensky, M., and J. Logel. 1983. Inhibition of degradation of 3-hydroxy-3-methylglutaryl coenzyme A reductase by mevinolin. *J. Biol. Chem.* 258:8547–8549.

46. Brown, M. S., J. R. Faust, J. L. Goldstein, I. Kaneko, and A. Endo. 1978. Induction of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in human fibroblasts incubated with compactin (ML-236B), a competitive inhibitor of the reductase. *J. Biol. Chem.* 253:1121–1128.

47. Kaneko, I., Y. Hazama-Shimada, and A. Endo. 1978. Inhibitory effects on lipid metabolism in cultured cells of ML-236B, a potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Eur. J. Biochem.* 87:313–321.

48. Maltese, W. A., B. A. Reitz, and J. J. Volpe. 1981. Effects of prior sterol depletion on neurite outgrowth in neuroblastoma cells. *J. Cell. Physiol.* 108:475–482.

49. Pons, G., R. F. O'Dea, and B. L. Mirkin. 1982. Biological characterization of the C-1300 murine neuroblastoma: an *in vivo* neural crest tumor model. *Cancer Res.* 42:3719–3723.

50. Finklestein, J. Z., E. Arima, P. E. Byfield, J. E. Byfield, and E. W. Fonkalsrud. 1973. Murine neuroblastoma: a model of human disease. *Cancer Chemother. Rep.* 57:405–412.

51. Murphy, G. P., P. Williams, and B. Keogh. 1975. Chemotherapy of a murine neuroblastoma model. *J. Surg. Oncol.* 7:521–524.

52. Finklestein, J. Z., K. Tittle, J. Rubenstein, R. Meshnik, and J. Weiner. 1976. Combination chemotherapy in murine neuroblastoma. *Med. Pediatr. Oncol.* 2:279–289.