Mevalonate Prevents Lovastatin-Induced Apoptosis in Medulloblastoma Cell Lines

Wei Wang and Robert J.B. Macaulay

ABSTRACT: ***Background:*** 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) is a key rate-limiting enzyme in the mevalonate pathway, which generates precursors for cholesterol biosynthesis and the production of non-steroidal mevalonate derivatives that are involved in a number of growth-regulatory processes. We have reported that lovastatin, a competitive inhibitor of HMG-CoA reductase, not only inhibits medulloblastoma proliferation *in vitro*, but also induces near-complete cell death via apoptosis. The present study explores some of the pathways which may be involved in lovastatin-induced apoptosis. ***Methods:*** Medulloblastoma cell lines were exposed *in vitro* to lovastatin with or without mevalonate, and document the effects using morphology, flow cytometry, DNA electrophoresis and Northern analysis. ***Results:*** 1) Mevalonate prevents apoptosis when co-incubated with lovastatin, or when administered to lovastatin-pretreated cells. 2) Mevalonate restores the lovastatin-arrested cell cycle, allowing S phase entry. 3) Mevalonate does not prevent lovastatin-induced apoptosis after a critical duration of lovastatin pretreatment. For cell lines Daoy and UW228 this was 24 hours, and for D283 Med and D341 Med it was 48 hours. 4) Increases in HMG-CoA reductase mRNA levels induced by lovastatin are abrogated by co-incubation with lovastatin and mevalonate. ***Conclusions:*** These results confirm that lovastatin inhibition of this enzyme results in blockage of the mevalonate pathway, and that such a block is a critical step in the mechanism of lovastatin-induced apoptosis.

RÉSUMÉ: Le mévalonate prévient l'apoptose induite par la lovastatine dans des lignées cellulaires de médulloblastome. ***Introduction:*** La 3-hydroxy-3-méthylglutaryl coenzyme a réductase (HMG-CoA réductase) est une enzyme clé, qui a un rôle d'étape cinétiquement limitante dans la voie du mévalonate et qui génère des précurseurs pour la biosynthèse du cholestérol et la production de dérivés non stéroïdiens du mévalonate impliqués dans certains processus de régulation de la croissance. Nous avons rapporté que la lovastatine, un inhibiteur compétitif de l'HMG-CoA réductase, non seulement inhibe la prolifération du médulloblastome in vitro, mais également induit presque complètement la mort cellulaire via l'apoptose. Cette étude explore certaines des voies qui pourraient être impliquées dans l'apoptose induite par la lovastatine. ***Méthodes:*** Les effets de l'exposition in vitro de lignées de cellules de médulloblastome à la lovastatine avec ou sans mévalonate ont été évalués par des études morphologiques, par cytométrie de flux, électrophorèse de l'ADN et analyse de Northern. ***Résultats:*** 1) Le mévalonate prévient l'apoptose quand les cellules sont coincubées avec la lovastatine ou quand il est administré à des cellules prétraitées par la lovastatine. 2) Le mévalonate rétablit le cycle cellulaire interrompu par la lovastatine, permettant une entrée en phase S. 3) Le mévalonate ne prévient pas l'apoptose induite par la lovastatine après un temps de prétraitement critique par la lovastatine. Ce temps de prétraitement était de 24 heures pour les lignées cellulaires Daoy et UW228, et de 48 heures pour les lignées D283 Med et D341 Med. 4) L'augmentation des niveaux d'ARNm de l'HMG-CoA réductase induite par la lovastatine est abolie par la coincubation avec la lovastatine et le mévalonate. ***Conclusions:*** Ces résultats confirment que l'inhibition de cette enzyme par la lovastatine provoque un blocage de la voie métabolique du mévalonate et que ce blocage est une étape critique dans le mécanisme de l'apoptose induite par la lovastatine.

Can. J. Neurol. Sci. 1999; 26: 305-310

Cancer is the second most frequent cause of death in children under 15 years of age, and tumors of the CNS following leukemia are the most common type of cancer to affect children.[1] Medulloblastoma, a primitive neuroectodermal tumor of the cerebellum,[2] accounts for about 20% of childhood intracranial tumors.[3] The prognosis of medulloblastomas is unpredictable for individual patients, with 50-70% survival after five years.[4,5] The mortality of recurrent medulloblastomas approaches 100%.[6] Radiation therapy is standard adjuvant therapy for medulloblastomas, but is not administered to patients less than three years old due to the deleterious effects on intellectual development.[7,8] The efficacy of adjuvant chemotherapy has also

From the Department of Pathology, College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada

RECEIVED FEBRUARY 22, 1999. ACCEPTED IN FINAL FORM MAY 26, 1999.

Reprint requests to: Rob Macaulay, Department of Pathology, QEII Health Sciences Center, Room 738 Mackenzie Building, 5788 University Avenue, Halifax, NS B3H 1V8

been assessed for childhood medulloblastomas. A beneficial effect of chemotherapy has recently been documented, but whether this will be accompanied by improvements in the long-term prognosis for intellectual development remains to be seen.[4,5] In addition, chemotherapy may result in dose-limiting toxicity and may induce expression of drug resistance genes.[9] The aggressiveness and resistance to conventional therapy of many medulloblastomas have thus led to the investigation of alternative therapeutic strategies.

Lovastatin, an inhibitor of HMG-CoA reductase, blocks the mevalonate pathway, reducing cholesterol biosynthesis as well as the production of non-steroidal mevalonate derivatives.[10] By uncertain mechanisms, this interrupts cellular proliferation, a phenomenon which has been exploited in studying cell division and proteins such as the cyclins.[11] Lovastatin has been suggested as a potential anticancer drug[12-15] and is known to perturb major signaling pathways by inhibiting isoprenylation of signal transduction proteins such as p21 ras.[14] Such proteins are then unable to anchor to plasma or nuclear membranes, abrogating their mitogenic or oncogenic activity.[16] Lovastatin has been shown to block signal transduction through the platelet-derived growth factor, epidermal growth factor, insulin-like growth factor, insulin receptor, and lipopolysaccharide pathways.[17] Lovastatin has exhibited potential as an anti-neoplastic agent, causing growth arrest and neuronal differentiation in neuroblastoma cells,[18] blocking cell cycling in G1 and at G2/M for a variety of tumor cell lines grown *in vitro*,[19] as well as suppressing the growth of human leukemia[20] and murine neuroblastoma *in vivo*.[21]

In addition to its inhibitory effects on tumor cell proliferation, lovastatin has been shown to induce apoptosis in four human medulloblastoma cell lines,[22] human acute T-cell leukemia,[23] HL-60 promyelocytic cells,[24] and human malignant glioma cells.[25] However, the signals and pathways involved in lovastatin-induced apoptosis remain elusive. Thus, we investigated whether the inhibition of mevalonate production is a critical step for lovastatin-induced apoptosis in medulloblastoma cell lines, and how mevalonate synthesis is physiologically regulated in these cells.

Materials and Methods

Unless stated otherwise, reagents were obtained from Gibco BRL.

Cells: Daoy (ATCC HTB-186), D283 MED (ATCC HTB-185) and D341 MED (ATCC HTB-187) were all obtained from the ATCC (American type culture collection), and UW228[26] was a gift from Dr. J Silber. These cells were cultured in D-MEM (Dulbecco's modified Eagle's medium)/F12 nutrient mixture with 10% fetal calf serum, L-glutamine and antibiotics in a humidified atmosphere of 5% CO2 at 37°C.

Primers: Based on the HMG-CoA reductase 3' flank sequence[27] primer pair GGCCTCTCTGAAGAAATAGCCTGC-GGAGAT (sense primer) and CCATGCAGACTCCTCAGAT-CTGAACACAGT (antisense primer) were used to amplify 525 bp of HMG-CoA reductase cDNA. Antisense primer, ACCACCATGGAGAAGGCTGG, was used in combination with the sense primer CTCAGTGTAGCCCAGGATGC to amplify 528 bp of the housekeeping gene GAPDH cDNA; this primer pair spans 3 introns (#5, 6 and 7) of the GAPDH gene sequence.[28]

Lovastatin and Mevalonate Treatment: The pro-drug form (lactone) of lovastatin was from Merck Research Laboratories, and converted to its active form (dihydroxy open acid) by using NaOH.[15] Medulloblastoma cells were treated with lovastatin as previously described[22] when they grew to subconfluency in flasks.

To assess the effects of mevalonate, cells were first treated for different time periods with lovastatin alone; they were then co-incubated with 20 μM of lovastatin and 2 mM of mevalonate (Sigma) together. The duration of lovastatin pretreatment was varied to establish a time window after which mevalonate was unable to restore cell viability. The duration of coadministration of the two agents varied from 36 to 96 hrs, according to the duration required to induce apoptosis with lovastatin alone.[22] The medium containing lovastatin and mevalonate was replaced every 48 hours where necessary. When replacing medium, the floating cells were aspirated, spun and returned to the original flasks. After treatment for different time periods with lovastatin and mevalonate, the attached and floating cells were harvested together by centrifugation for various analytical studies. Experiments were repeated in triplicate.

Purification and Analysis of DNA: The DNA of lovastatin- and mevalonate-treated cells was extracted and analyzed on agarose gel as previously described.[22]

Flow Cytometry: Lovastatin- and mevalonate-treated cells were also analyzed by flow cytometry (Coulter(R) Epics(R)) as previously described.[22] The results of flow cytometry were analyzed by "overlapped peak" multicycle fitting option.

Synthesis of cDNA Probes: To prepare probes recognizing HMG-CoA reductase mRNA and GAPDH mRNA, their cDNA fragments were generated using RT-PCR. For reverse transcription, total human cellular RNA (1 μg) was used for cDNA synthesis. First-strand cDNA synthesis was carried out in 20 μl reaction containing 1 x PCR buffer, 1mM each of deoxynucleotide triphosphates (dNTP) (Pharmacia), 20 units placental ribonuclease inhibitor (RNAguard, Pharmacia), 160 units of MuLV-reverse transcriptase (Pharmacia) and 100 pmol of random hexamer oligodeoxynucleotides (Pharmacia).[29] After preincubation at 21°C for 10 minutes, the reaction proceeded for one hour at 42°C, followed by 95°C for 10min. For cDNA amplification, PCR was performed on a Perkin-Elmer GeneAmp PCR System 2400. The reaction contained 2 μl of RT reaction products as template DNA, 1 x PCR buffer, 180 μM of each of dNTP, 20 pmol of each antisense and sense primer pair of either HMG-CoA reductase or GAPDH, and 2.5 units of *Taq* DNA polymerase. Amplification conditions for HMG-CoA reductase cDNA amplification were 94°C for 90 seconds, 60°C for 45 seconds, and 72°C for 60 seconds for 32 cycles. RT-PCR amplified cDNA fragments of GAPDH and HMG-CoA reductase were labeled with [^{32}P]dCTP using the Random Primed DNA Labeling Kit (Boehringer Mannheim) according to instructions of the manufacturer.

RNA Isolation and Northern Blot Analysis: Total cellular RNA was extracted from cultured medulloblastoma cells using the acid guanidinium-phenol-chloroform method with the TriZol reagent. Lovastatin- and mevalonate-treated adherent and nonadherent cells (10^6 to 10^7) were washed three times with RNase-free PBS, lysed in 1 ml of TriZol reagent, and then RNA was isolated according to the manufacturer's instructions. Total RNA (30 μg) was electrophoresed in 1.2% agrose/1.8%

https://doi.org/10.1017/S0317167100000433 Published online by Cambridge University Press

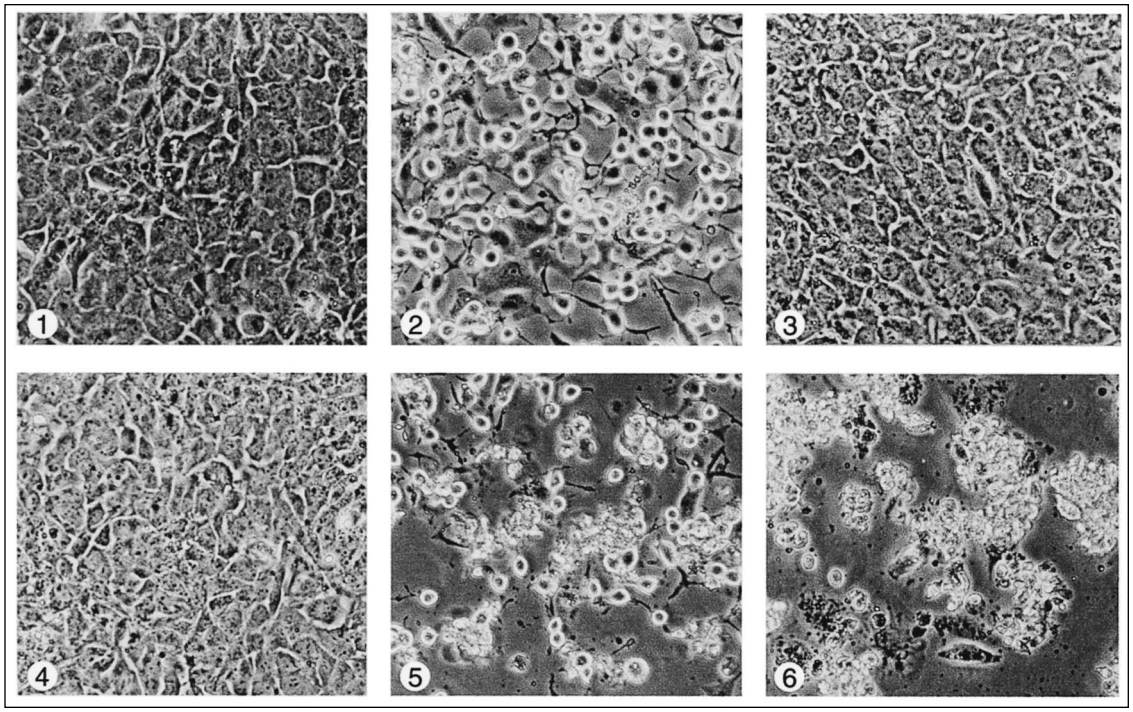

Figure 1: *Morphological Changes to Lovastatin and Mevalonate. 1) Daoy cells grow as a flattened monolayer in culture. 2) After exposure to lovastatin (20 μM) for 24 hr, cells begin to detach and round up. 3) Mevalonate (2 mM) rescues Daoy cells treated for 24 hr with lovastatin, restoring original morphology. 4) No change is seen if lovastatin and mevalonate are co-administered for 48 hr. 5) Lovastatin alone induces marked morphologic changes after 48 hr, indicative of apoptosis. 6) After 48 hr of lovastatin treatment, mevalonate is unable to restore original morphology. UW228, D341 Med and D283 Med cells showed similar results, although with different time points depending on the sensitivity of the cell line to lovastatin-induced apoptosis.*

formaldehyde gel and transferred to a Hybond-N+ membrane (Amersham) with 1.5 M sodium chloride, 0.15 M sodium citrate (10 x SSC) as transfer buffer. Membranes were baked for two hours at 80°C, prehybridized in Hybrisol solution (Oncor) at 42°C for two hours, and then hybridized at 42°C for 16-18 hours in the same buffer containing 10^6 cpm/ml heat-denatured HMG-CoA reductase cDNA probe. Membranes were washed twice with 2 x SSC/0.1% SDS at room temperature for 15 minutes, followed by 4 x 15 minute washes in 0.1 x SSC/0.1% SDS at 52°C prior to autoradiography. Autoradiograms were exposed at -70°C. After autoradiography membranes were boiled in RNase-free water for 2 x 10 minutes to strip the probe, rehybridized with GAPDH cDNA probe, and washed twice with 2 x SSC/0.1% SDS at room temperature for 15 minutes, and twice for 30 minutes with 0.1 x SSC/0.1% SDS at 65°C, prior to autoradiography.

Results

Mevalonate Prevents Lovastatin-induced Morphological Changes

Morphological changes of medulloblastoma cells treated with lovastatin have previously been reported.[22] Each cell line shows a different sensitivity to lovastatin: shrinkage of Daoy and UW228 cells commences after 12-24 hrs of treatment with lovastatin, while D283 Med and D341 Med shrinkage and complete detachment requires about 72 hrs.[22] In all tested cell lines, these morphological changes were reversible after the cells were co-

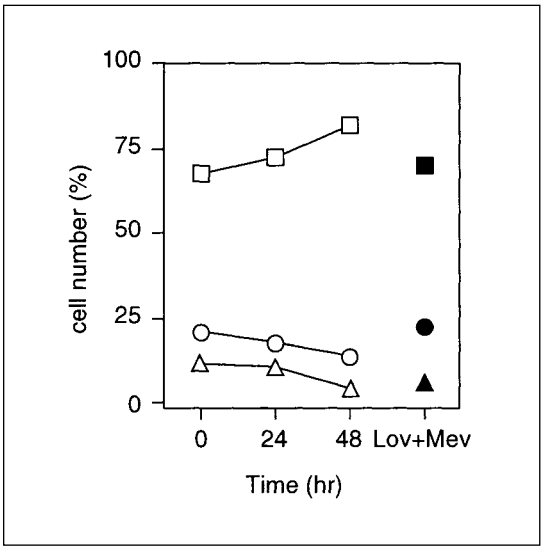

Figure 2: *Cell Cycle Changes in Daoy Treated With Lovastatin and Mevalonate. Flow cytometric analysis reveals an increasing proportion of cells in G1 (open squares), while the proportion of cells in S (open circles) and G2 (open triangles) decreases after 24 and 48 hrs of lovastatin (20 μM). When lovastatin-treated cells (24 hr) were replenished with a mixture of lovastatin and mevalonate (2 mM), cell cycle parameters approached control values by 36 hr (solid symbols). Similar results were obtained for UW228, D283 Med and D341 Med; in addition, for D341 and D283 Med there appeared to be G2 block, which was also abrogated after co-treatment with mevalonate.*

https://doi.org/10.1017/S0317167100000433 Published online by Cambridge University Press

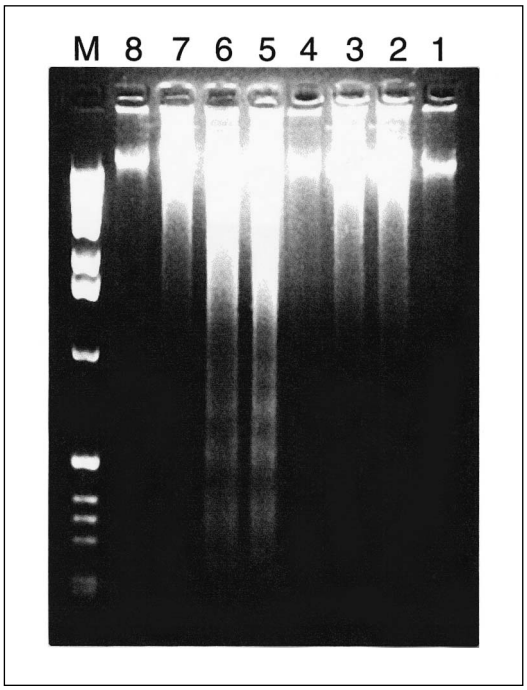

Figure 3: *DNA Laddering in Daoy after Lovastatin and Mevalonate Treatment. Extracted DNA is intact at: Time 0 (lane 1); after lovastatin (20 μM) x 24 hr (lane 2); after lovastatin x 24 hr followed by lovastatin and mevalonate (2 mM) x 48 hr (lane 3); and after lovastatin and mevalonate co-incubation x 24 hr (lane 4). Lovastatin x 48 hr induces DNA laddering (lane 5). Mevalonate cannot rescue cells pre-treated with lovastatin alone x 48 hr (lane 6). No laddering is seen following lovastatin and mevalonate co-incubation x 48 hr without lovastatin pre-treatment (lane 7); DNA from untreated cells incubated for 48 hr is also intact (lane 8); M: molecular weight marker. DNA from UW228, D283 Med and D341 Med cells showed similar findings.*

incubated with lovastatin and mevalonate, but only if the duration of lovastatin pre-treatment was limited to a critical time window (Figure 1). Mevalonate could not reverse the morphological changes in those cells that were treated with lovastatin for longer durations, and were showing apoptotic bodies (Figure 1). Non-pretreated cells failed to show morphological changes when they were treated with lovastatin and mevalonate.

Mevalonate Prevents Lovastatin-induced Flow Cytometric Changes

After pre-treatment with lovastatin for limited durations, MB cells were again exposed to lovastatin and mevalonate for durations equivalent to those which induced flow cytometric evidence of apoptosis when lovastatin was administered alone.[22] All four MB cell lines failed to exhibit an 'apoptosis peak' under these conditions (data not shown). Cell cycle changes induced by lovastatin were also abolished; thus, G1 arrest in all four cell lines was overcome by mevalonate, as was G2 arrest in D283 Med and D341 Med (Figure 2). In contrast, for cells treated with lovastatin for longer time periods the 'apoptosis peak' remained despite co-incubation with lovastatin and mevalonate (data not shown). Cell cycle changes and apoptosis peaks did not develop when cells were treated with lovastatin and mevalonate together without lovastatin pre-treatment.

Mevalonate Prevents Lovastatin-induced DNA Laddering

DNA fragmentation characteristic of apoptosis follows lovastatin treatment of medulloblastoma cell lines *in vitro*.[22] Mevalonate prevented lovastatin induced DNA fragmentation when mevalonate was administrated to cells pretreated with lovastatin for limited durations (Figure 3), but failed to reverse lovastatin-induced DNA fragmentation after longer lovastatin pretreatments cells (Figure 3 lanes 5&6). Co-administration of lovastatin and mevalonate failed to induce DNA laddering (Figure 3 lanes 4&7), indicating that mevalonate can prevent lovastatin-induced apoptosis if administered before the cell death program has been executed. For each cell line, a critical time window was identified beyond which mevalonate can no longer salvage lovastatin-treated cells; for the attached cell lines Daoy and UW228 it was between 24 and 48 hours, whereas it was considerably longer for the partially attached cell lines D283 Med and D341 Med, between 48 and 96 hours.

Physiological Regulation of HMG-CoA Transcription.

[^{32}P]-labeled human HMG-CoA reductase cDNA probe was hybridized with electrophoresed total cellular RNAs from lovastatin-treated medulloblastoma cells. Levels of HMG-CoA

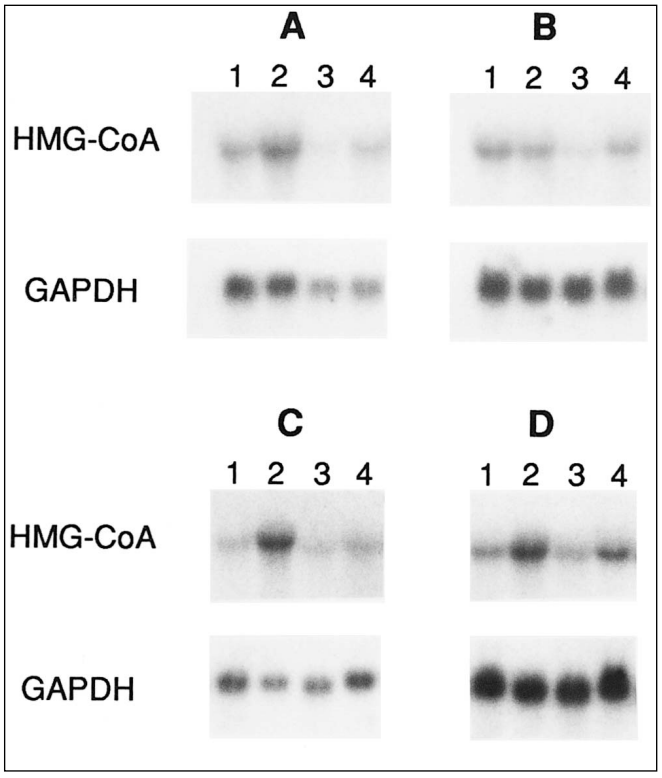

Figure 4: *HMG-CoA transcription in medulloblastoma cells treated with 20 μM lovastatin and 2 mM mevalonate. Note the upregulation of HMG-CoA following lovastatin administration in Daoy, D283 Med and D341 Med, and the marked down-regulation accompanying mevalonate coadministration in all four cell lines. A: Daoy; B: UW228; C: D283 Med; and D: D341 Med Lane 1: untreated; lane 2: lovastatin (24 h for Daoy and UW228; 48 hr for D283 med and D341 Med); lane 3: lovastatin and mevalonate co-incubation x 36 hr, after lovastatin pre-treatments (see lane 2); and lane 4: lovastatin and mevalonate co-incubation (24 hr for Daoy and UW 228; 48 hr for D283 Med and D341 Med) without lovastatin pretreatment. GAPDH expression reveals minimal variability in loading.*

https://doi.org/10.1017/S0317167100000433 Published online by Cambridge University Press

reductase expression in Daoy, D283 Med and D341 Med were increased after medulloblastoma cells were treated with up to 40 mM lovastatin (Figure 4A, C and D, lane 2 in each). Curiously, HMG-CoA reductase expression in the adherent cell line UW228 was not altered by lovastatin (Figure 4B, lane 2). In addition, more pronounced increases were seen in the partially attached cell lines D283 Med and D341 Med, compared with the other adherent cell line Daoy. In all four cell lines, expression of HMG-CoA reductase mRNA was reduced below baseline when cells pretreated with lovastatin for limited durations were then incubated with lovastatin and mevalonate (Figure 4A-D, lane 3 in each). Fresh cells co-incubated with both lovastatin and mevalonate showed similar expression of HMG-CoA reductase mRNA as untreated cells (Figure 4A-D, lanes 1&4 in each).

Discussion

Several studies have demonstrated the ability of lovastatin to inhibit proliferation of tumor cells *in vitro*,[19] and induce cell death via apoptosis[22-25,30] raising the possibility of using lovastatin clinically as an anti-tumor drug. However, systematic investigation into the mechanism of lovastatin-induced apoptosis has not been resolved. We present data showing alterations in HMG-CoA reductase which confirm that lovastatin inhibits mevalonate production. We have also compiled morphological, cell cycle and DNA fragmentation data which demonstrate that mevalonate overcomes lovastatin-induced apoptosis. We thus conclude that blocking mevalonate production is a critical step in the mechanism of lovastatin-induced apoptosis, and that mevalonate administration averts lovastatin-induced apoptosis before the apoptosis sequence is irreversible.

Since blocking the production of mevalonate is responsible for lovastatin-induced apoptosis, it follows that depletion of specific mevalonate derivatives must be involved in this phenomenon. One important requirement may be the inhibition of isoprenylation of particular cellular proteins. A number of important signalling proteins require isoprenylation in order to localize to the cell membrane and interact with other components of various signal transduction pathways.[16] Mutant p21 ras[13] and heterotrimeric G protein[31] are well-studied isoprenylated signaling proteins which contribute to tumor growth; however, whether the inhibition of isoprenylation of these or other signaling proteins is responsible for lovastatin-induced apoptosis is not clear.[32]

HMG-CoA reductase is controlled by several feedback-regulation mechanisms.[12] Our data indicate that transcription of the HMG-CoA reductase gene is increased following lovastatin administration *in vitro* because of decreased mevalonate production. HMG-CoA reductase expression is maintained at normal levels when mevalonate and lovastatin are co-administered. However, this feedback regulation of HMG-CoA reductase varies among the four medulloblastoma cell lines tested, in that expression levels in Daoy were less affected than in D283 Med and D341 Med, while UW228 showed changes in expression only if pre-treated with lovastatin. This indicates that the capacity of the adherent cell lines to upregulate HMG-CoA reductase may be limited, compared to the partially attached cell lines. We speculate that the limited capacity to upregulate HMG-CoA reductase in the adherent cell lines may confer increased sensitivity to lovastatin, since shorter treatment times were sufficient to induce apoptosis in Daoy and UW228 compared with D283 Med and D341 Med.

Different genetic characteristics among the four medulloblastoma cell lines may also affect their sensitivity to lovastatin. For example, overexpression of the *c-myc* oncogene is detectable in D283 Med and D341 Med.[33] Expression of the *myc* transcription factors is important for cell proliferation;[34] *myc* has also been implicated in the induction of apoptosis under certain conditions which cause growth arrest, such as growth factor and serum deprivation.[35-37] A number of reports have focussed on the oncogenic activity of *myc* proteins. *Myc* interacts with the retinoblastoma protein (pRB) and is able to override pRB-induced cell cycle arrest. Cell proliferation was induced when *myc* was expressed in the presence of certain growth promoting cytokines such as IL-2. Overexpression of *myc* results in uncontrolled cell proliferation.[38] In our experimental system, cells were administrated with lovastatin in standard growth medium containing serum. Thus, it is possible that overexpression of *c-myc* in D283 Med and D341 Med confers relative resistance to lovastatin-induced apoptosis, a possibility which is currently being investigated in our laboratory.

We have demonstrated that lovastatin-treated cells can be diverted from the apoptosis pathway by mevalonate administration, but only if mevalonate is provided within a critical time period. Medulloblastoma cells treated with lovastatin for time periods beyond this 'window of opportunity' were apparently committed to die. A number of signals involving a variety of distinct pathways appear to trigger a common apoptosis pathway, yielding characteristic morphologic changes, DNA laddering and a flow cytometric 'apoptosis peak'. The common end stage for these different triggers appears to be the activation of ICE-related proteases, intracellular proteases which play a critical role in the execution of apoptosis. Inactive precursors of ICE-related proteases are cleaved at aspartate residues to become active, resulting in apoptosis.[39-41] We are pursuing the possibility that ICE-related protein cleavage characterizes lovastatin-induced apoptosis of medulloblastoma cells.[32] Interfering directly with the function of ICE-related proteases or their precursors might inhibit cell death, and it is conceivable that mevalonate inhibition of lovastatin-induced apoptosis is simply the result of such a phenomenon. However, mevalonate and its derivatives are not known to interact with ICE-related proteases, and we consider this to be an unlikely explanation for the presented data.

Acknowledgements

We thank Mr. Bob van den Beuken and Mr. Todd Reichart for excellent photographic assistance, Mrs. Connie McGregor for technical assistance, and Drs LE Becker, H Yeger and J Dimitroulakous for support and advice. This study was partially funded by the Health Services Utilization and Research Commission of Saskatchewan, and partially by the Brain Tumour Research Foundation of Canada.

References

1. Boring CC, Squires TS and Tong T. Cancer statistics, 1991 [published erratum appears in CA Cancer J Clin 1991 Mar-Apr;41(2):111]. Ca Cancer J Clin 1991; 41: 19-36.
2. Jay V and Becker, LE. Brain tumors. Curr Opin Neurol Neurosurg 1990; 3: 934-942.
3. Schoenberg BS, Schoenberg DG, Christine BW and Gomez MR.

The epidemiology of primary intracranial neoplasms of childhood. Mayo Clin Proc 1976; 51: 51-56.
4. Packer RJ. Outcome for children with medulloblastoma treated with radiation and cisplatin, CCNU and vincristine chemotherapy. J Neurosurg 1994; 81: 690-698.
5. Rorke LB, Trojanowski JQ, Lee VM et al. Primitive neuroectodermal tumors of the central nervous system. Brain Pathol 1997; 7: 765-84.
6. Torres CF, Rebsamen S, Silber JH, et al. Surveillance scanning of children with medulloblastoma. N Engl J Med 1994; 330: 892-5.
7. Tomlinson FH, Scheithauer BW, Meyer FB et al. Medulloblastoma: I. Clinical, diagnostic, and therapeutic overview. J Child Neurol 1992; 7: 142-55.
8. Nishiyama K, Funakoshi S, Izumoto S et al. Long-term effects of radiation for medulloblastoma on intellectual and physical development. A case report of monozygotic twins. Cancer 1994; 73: 2450-5.
9. Tishler DM, Weinberg KI, Sender LS et al. Multidrug resistance gene expression in pediatric primitive neuroectodermal tumors of the central nervous system. J Neurosurg 1992; 76: 507-12.
10. Tobert JA, Hitzenberger G, Kukovetz WR et al. Rapid and substantial lowering of human serum cholesterol by mevinolin (MK-803), an inhibitor of hydroxymethylglutaryl-coenzyme A reductase. Atherosclerosis 1982; 41: 61-5.
11. Poon RY, Toyoshima H and Hunter T. Redistribution of the CDK inhibitor p27 between different cyclin.CDK complexes in the mouse fibroblast cell cycle and in cells arrested with lovastatin or ultraviolet irradiation. Mol Biol Cell 1995; 6: 1197-213.
12. Goldstein JL and Brown MS. Regulation of the mevalonate pathway. Nature 1990; 343: 425-30.
13. Hancock JF, Magee AI, Childs JE and Marshall CJ. All ras proteins are polyisoprenylated but only some are palmitoylated. Cell 1989; 57: 1167-77.
14. O'Donnell MP, Kasiske BL, Kim Y et al. The mevalonate pathway: importance in mesangial cell biology and glomerular disease. Miner Electrolyte Metab 1993; 19: 173-9.
15. Keyomarsi K, Sandoval L, Band V and Pardee AB. Synchronization of tumor and normal cells from G1 to multiple cell cycles by lovastatin. Cancer Res 1991; 51: 3602-9.
16. Khosravi FR, Cox AD, Kato K and Der CJ. Protein prenylation: key to ras function and cancer intervention? Cell Growth Differ 1992; 3: 461-9.
17. Law RE, Stimmel JB, Damore MA et al. Lipopolysaccharide-induced NF-kappa B activation in mouse 70Z/3 pre-B lymphocytes is inhibited by mevinolin and 5'-methylthioadenosine: roles of protein isoprenylation and carboxyl methylation reactions. Mol Cell Biol 1992; 12: 103-11.
18. Dimitroulakos J, Pienkowska M, Squire J et al. Differential display of mRNAs using PCR (DD-PCR) to identify genes involved in the growth and differentiation of human neuroblastomas (abstract). Brain Pathol 1994; 4: 427.
19. Newman A, Clutterbuck RD, Powles RL and Millar JL. Selective inhibition of primary acute myeloid leukemia cell growth by lovastatin [corrected and republished in Leukemia 1994 Nov;8(11):2022-9]. Leukemia 1994; 8: 274-80.
20. Dimitroulakos J, Nohynek D, Backway KL et al. Increased sensitivity of acute myeloid leukemias to lovastatin-induced apoptosis: a potential therapeutic approach. Blood 1999; 93: 1308-18.
21. Maltese WA, Defendini R, Green RA et al. Suppression of murine neuroblastoma growth in vivo by mevinolin, a competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. J Clin Invest 1985; 76: 1748-54.
22. Macaulay RJB, Wang W, Dimitroulakos J et al. Lovastatin-induced apoptosis of human medulloblastoma cell lines in vitro. J Neuro-Oncol 1999; 42: 1-11.
23. Bansal N, Houle AG and Melnykovych G. Comparison of dexamethasone and lovastatin (mevinolin) as growth inhibitors in cultures of T-cell derived human acute leukemia lines (CEM). Leuk Res 1989; 13: 875-82.
24. Perez SD and Mollinedo F. Inhibition of isoprenoid biosynthesis induces apoptosis in human promyelocytic HL-60 cells. Biochem Biophys Res Commun 1994; 199: 1209-15.
25. Jones KD, Couldwell WT, Hinton DR et al. Lovastatin induces growth inhibition and apoptosis in human malignant glioma cells. Biochem Biophys Res Commun 1994; 205: 1681-7.
26. Keles GE, Berger MS, Srinivasan J et al. Establishment and characterization of four human medulloblastoma-derived cell lines. Oncol Res 1995; 7: 493-503.
27. Ramharack R, Tam SP and Deeley RG. Characterization of three distinct size classes of human 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA: expression of the transcripts in hepatic and nonhepatic cells. DNA Cell Biol 1990; 9: 677-90.
28. Ercolani L, Florence B, Denaro M and Alexander M. Isolation and complete sequence of a functional human glyceraldehyde-3-phosphate dehydrogenase gene. J Biol Chem 1988; 263: 15335-41.
29. Wong H, Anderson WD, Cheng T and Riabowol KT. Monitoring mRNA expression by polymerase chain reaction: the "primer-dropping" method. Anal Biochem 1994; 223: 251-8.
30. Dimitroulakos J and Yeger H. HMG-CoA reductase mediates the biological effects of retinoic acid on human neuroblastoma cells: lovastatin specifically targets P-glycoprotein-expressing cells. Nat Med 1996; 2: 326-33.
31. Finegold AA, Schafer WR, Rine J et al. Common modifications of trimeric G proteins and ras protein: involvement of polyisoprenylation. Science 1990; 249: 165-9.
32. Wang W and Macaulay RJB. Apoptosis of medulloblastoma cells in vitro follows inhibition of farnesylation using manumycin A. Int J Cancer 1999; 82: 430-434.
33. Pietsch T, Scharmann T, Fonatsch C et al. Characterization of five new cell lines derived from human primitive neuroectodermal tumors of the central nervous system. Cancer Res 1994; 54: 3278-87.
34. Green DR, Mahboubi A, Nishioka W et al. Promotion and inhibition of activation-induced apoptosis in T-cell hybridomas by oncogenes and related signals. Immunol Rev 1994; 142: 321-42.
35. Askew DS, Ashmun RA, Simmons BC and Cleveland JL. Constitutive c-myc expression in an IL-3-dependent myeloid cell line suppresses cell cycle arrest and accelerates apoptosis. Oncogene 1991; 6: 1915-22.
36. Evan GI, Wyllie AH, Gilbert CS et al. Induction of apoptosis in fibroblasts by c-myc protein. Cell 1992; 69: 119-28.
37. Hermeking H and Eick D. Mediation of c-Myc-induced apoptosis by p53. Science 1994; 265: 2091-3.
38. Duffy MJ. Cellular oncogenes and suppressor genes as prognostic markers in cancer. Clin Biochem 1993; 26: 439-47.
39. Munday NA, Vaillancourt JP, Ali A et al. Molecular cloning and pro-apoptotic activity of ICErelII and ICErelIII, members of the ICE/CED-3 family of cysteine proteases. J Biol Chem 1995; 270: 15870-6.
40. Nicholson DW, Ali A, Thornberry NA et al. Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. Nature 1995; 376: 37-43.
41. Sleath PR, Hendrickson RC, Kronheim SR et al. Substrate specificity of the protease that processes human interleukin-1 beta. J Biol Chem 1990; 265: 14526-8.