*Biochemical Pharmacology*, Vol. 45, No. 11, pp. 2203–2208, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00


# PRAVASTATIN INHIBITED THE CHOLESTEROL SYNTHESIS IN HUMAN HEPATOMA CELL LINE Hep G2 LESS THAN SIMVASTATIN AND LOVASTATIN, WHICH IS REFLECTED IN THE UPREGULATION OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE AND SQUALENE SYNTHASE

LOUIS H. COHEN,* ARLÈNE VAN VLIET, LOES ROODENBURG, LUCRES M. C. JANSEN and MARIEKE GRIFFIOEN

Gaubius Laboratory IVVO-TNO, P.O. Box 430, 2300 AK Leiden, The Netherlands

(*Received* 9 *November* 1992; *accepted* 16 *March* 1993)

**Abstract**—The possible difference between lovastatin (mevinolin, MK-803), simvastatin (MK-733) and pravastatin (CS-514), all chemically-related competitive inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, were tested in the human hepatoma cell line Hep G2, which is often used as a model for the human hepatocyte. After an 18-hr incubation of the cells with the drugs, pravastatin ($IC_{50}$ = 1900 nM) was less potent than simvastatin and lovastastin ($IC_{50}$ = 34 and 24 nM, respectively) in inhibiting the sterol synthesis. As a consequence of this inhibition, the HMG-CoA reductase mRNA levels and squalene synthase activity, both negatively-regulated by sterols, were increased equally by simvastatin and lovastatin, whereas the induction by pravastatin was much less. In contrast, there were fewer differences between the compounds in inhibiting HMG-CoA reductase activity, when assayed directly in Hep G2 cell homogenates ($IC_{50}$ values = 18, 61 and 95 nM for simvastatin, lovastastin and pravastatin, respectively). Moreover, in experiments with human hepatocytes in primary culture the $IC_{50}$ values for inhibition of the cholesterol synthesis by simvastatin and pravastatin were of the same order of magnitude (23 and 105 nM, respectively). The results are therefore explained as follows: the three drugs act in the same way within the Hep G2 cell in terms of inhibiting HMG-CoA reductase and their subsequent effect on the feedback regulation of the cholesterol synthesis, i.e. increasing squalene synthase and HMG-CoA reductase mRNA. However, pravastatin seems to be less able to enter the cells compared with simvastatin and lovastatin, possibly because of the higher hydrophobicity of the latter compounds. The observation with human hepatocytes suggests that in Hep G2 cells a specific hepatic transporter is missing. On one hand the human hepatoma cell line Hep G2 has proved to be a good model for the study of the feedback regulation of enzymes of the cholesterol biosynthetic pathway such as HMG-CoA reductase and squalene synthase, but, on the other hand seems to be less suitable as a model for the study of specific uptake of drugs, e.g. the vastatins, in human hepatocytes.

The search for new cholesterol-lowering drugs induced the development of different analogues of compactin (ML-23B, CS-500) [1], a competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA†) reductase (EC 1.1.1.34), which is the major rate limiting enzyme of sterol synthesis in eukaryotic cells. During the last years these analogues such as lovastatin (MK-803, mevinolin) [2], simvastatin (MK-733) [3] and pravastatin (CS-514) [4], have been taken by many people in order to lower their serum cholesterol levels [5].

Although their efficacy in lowering serum cholesterol levels in men is of the same order of magnitude (simvastatin is about twice as potent as pravastatin and lovastatin; see Ref. 5) several authors have reported a large difference in the potency of the three drugs to inhibit the cholesterol synthesis in animal extrahepatic tissue *in vivo* and cells in culture compared to that in hepatic tissue and cells [4, 6, 7]. Pravastatin, the more hydrophilic one, compared to simvastatin and lovastatin [8] had a reduced ability to inhibit cholesterol synthesis in extrahepatic tissue, whereas similar inhibition was observed for the three drugs in hepatic cells. These differences have also been observed in human fibroblasts [4] and human lens [9] *in vitro* but no data have been published on this matter concerning human liver tissue.

The human hepatoma cell line Hep G2 still possesses a number of human hepatocyte characteristics [10] and therefore has been frequently used as a model for the human hepatocyte. We have been using this cell line as such a model system in order to study the feedback regulation of the cholesterol synthesis and of low density lipoprotein (LDL)-receptor activity. In these cells compactin, inhibiting the incorporation of [$^{14}$C]acetate into cholesterol, induced in a concentration-dependent manner, the LDL-receptor and the HMG-CoA reductase activity [11], HMG-CoA reductase mRNA levels [12] and

* Corresponding author. Tel. (31)71.181818; FAX (31)71.181904.

† Abbreviations: HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; DMEM, Dulbecco's modified Eagle's medium.

the squalene synthase (EC 2.5.1.21) activity [13]. From these results we concluded that the negative feedback regulation of these processes by mevalonate-derived products takes place in Hep G2 cells as well.

In the present study we investigated the inhibitory potency of the analogues of compactin, i.e. lovastatin, simvastatin and pravastatin on the cholesterol synthesis in intact Hep G2 cells in culture and directly on the HMG-CoA reductase activity in homogenates of this human hepatocyte-derived cell line. Further we checked whether the differences observed were sustained by comparable effects on the co-ordinate regulation of HMG-CoA reductase and squalene synthase. Some data on the potency of the vastatins in inhibiting the cholesterol synthesis in human hepatocytes in primary culture are presented as well.

## MATERIALS AND METHODS

*Materials.* Lovastatin (mevinolin, MK-803) and simvastatin (synvinolin, MK-703) were kind gifts from Merck, Sharp and Dohm (Rahway, NJ, U.S.A.) and pravastatin (CS-514) was donated by the Sankyo Co. (Tokyo, Japan). Stock solutions were made in ethanol. The open acid forms of lovastatin and simvastatin were freshly prepared before use as described previously [9].

cDNA probes coding for human HMG-CoA reductase and for human serum albumin were isolated from plasmids obtained from Dr K. L. Luskey (Dept of Molecular Genetics and Internal Medicine, University of Texas, Dallas, TX, U.S.A.) and from Dr H. Pannekoek (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) respectively, as described previously [12].

*Human hepatoma cell line Hep G2.* The cells were cultured in 10 $cm^2$ multiwell dishes in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% (w/v) foetal bovine serum. At 18 hr before harvesting, the medium was replaced with DMEM, supplemented with 1% (w/v) palmitate-loaded (10 $\mu$mol/g) albumin [14] and the open acid forms of the different inhibitors at concentrations indicated in the Results. Ethanol was added to all incubations upto 0.05% (v/v), which was present at the highest drug concentration used. At this concentration ethanol did not influence the parameters determined. Cells were incubated for 18 hr at 37° in a 5% $CO_2$ atmosphere.

*Human hepatocytes.* The procedure and conditions for the isolation and culture of human hepatocytes has been described by Kooistra *et al.* [15]. Twenty-four hours after seeding of 1–1.5 × $10^6$ of viable cells in 10 $cm^2$-wells the incubations were performed in Williams E medium supplemented with 10% heat-inactivated foetal bovine serum, 135 nM insulin, 50 nM dexamethasone, 100 IU/mL penicillin and 0.1 mg/mL of streptomycin, at 37° in a 5% $CO_2$/95% air atmosphere.

*Determination of the cholesterol synthesis.* Measurements of the sterol synthesis ([$^{14}$C]acetate incorporation into non-saponifiable lipids) in Hep G2 cells and in human hepatocytes in the absence or presence of different drug concentrations was performed according to a modification of a previously described method [14]. After the cells had been incubated for 1 hr with the medium containing the inhibitors [$^{14}$C]acetate (Amersham, Amersham, U.K.; sp. radioact. 56.2 mCi/mmol) was added (0.4 or 2 $\mu$Ci/well containing 1 mL of medium for Hep G2 or hepatocytes, respectively). The incubation was continued for 17 hr and then the medium was removed, the cells were lysed in 300 $\mu$L of 0.2 M NaOH and subsequently neutralized with 30 $\mu$L 2 M HCl. Media and cell lysates were stored at −20°. After thawing samples were taken for protein determination. Thereafter, total lipids were extracted from cell lysate and medium together, according to Bligh and Dyer [16], in the presence of 0.008% (w/v) butylated hydroxytoluene (Sigma Chemical Co. Poole, U.K.) as an antioxidant and 0.01 $\mu$Ci of [$^3$H]cholesterol (New England Nuclear, Stevenage, U.K.; sp. radioact. 24 Ci/mmol) as a recovery standard. After evaporating the chloroform from the lipid extract under $N_2$, saponification was conducted in 0.2 mL of ethanolic (96%) 0.5 M NaOH for 1.5 hr at 60°. After cooling, 0.2 mL of water was added and the non-saponifiable lipids were extracted with 2 × 0.5 mL of hexane. The combined hexane extracts were washed with 0.8 mL of ethanolic (48%) 0.25 M NaOH, followed by evaporation of the hexane under nitrogen. The $^{14}$C-radioactivity incorporated into the non-saponifiable lipids were determined in a Tri-carb liquid scintillation analyser (Packard), corrected for the recovery of [$^3$H]cholesterol and expressed as $^{14}$C-dpm/mg of cellular protein. Values are the average of duplicate cell incubations. The data presented in the figures are expressed as percentages of the control values, as means ± SEM, obtained from four to five separately performed Hep G2 cell-experiments and from three experiments using human hepatocytes.

*Isolation of RNA from Hep G2 cells.* RNA was isolated from Hep G2 cells according to the procedure described by Chomczynski and Sacchi [17]. In brief, cells from two 10 $cm^2$-wells were washed with phosphate-buffered saline (PBS) and harvested in 0.5 mL of 4 M guanidinium isothiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% (w/v) sarkosyl and 0.1 M $\beta$-mercaptoethanol. RNA was extracted from the cell lysates with phenol/chloroform/isoamyl alcohol and precipitated subsequently with isopropanol. After washing the pellets with 70% ethanol they were dissolved in 40 $\mu$L of 10 mM Tris–HCl (pH 7.0). The RNA concentration of each sample was determined spectrophotometrically. The yield of RNA was usually 100–150 $\mu$g.

*Determination of HMG-CoA reductase mRNA levels.* Using the cDNA probes as described above, the reductase mRNA levels were determined by northern blot hybridization. The albumin probe was used as an internal mRNA standard, while the albumin mRNA levels were not significantly influenced by the treatments of the cells (compare with Ref. 12). Five micrograms (which was within the range of linearity between mRNA concentration and hybridization signal) of total RNA from the different dishes were incubated with formamide in

gel running buffer at 55° for 15 min to denature RNA and then subjected to gel electrophoresis in formaldehyde agarose gels. After electrophoresis, RNA was transferred to Hybond N filters according to the instructions of the manufacturer. Prehybridization and hybridization were performed at 60° (1 mM EDTA; 7% sodium dodecyl sulphate; 0.25 M NaCl, 0.25 M $NaH_2PO_4/Na_2HPO_4$; pH 7.2) essentially as described elsewhere [18]. Hybridization was usually performed with 1 ng/mL probe labelled by the random-primer method (Multi-prime, Amersham, Houten, The Netherlands) with [$^{32}$P]dCTP to approximately $10^9$ cpm/$\mu$g DNA.

The filters were washed at a stringency of 0.1 × standard saline citrate and 1% sodium dodecyl sulphate twice for 15 min each at 65° [19]. The membranes were subsequently exposed to Amersham Hyperfilm-MP with an intensifying screen at −80°. For quantification of the relative amounts of mRNA on the autoradiograph a scan of the bands was made on a CS 910 Shimadzu scanner. The areas under the peaks were integrated and plotted with the aid of a United Technology Packard data processor. The values for the HMG-CoA reductase mRNA contents are expressed as HMG-CoA-reductase-probe-derived blackening (arbitrary) units divided by albumin-probe-derived blackening units.

*Assay of HMG-CoA reductase activity.* HMG-CoA reductase activity was determined in the Hep G2 cell homogenate essentially as described previously [11]. Cells were washed three times with cold phosphate-buffered saline (0.15 M NaCl/10 mM $Na_2HPO_4$/1.5 mM $KH_2PO_4$, pH 7.4) and once with 0.1 M potassium phosphate/0.1 M NaCl (pH 7.4), and were harvested by scraping them into assay buffer (0.1 M potassium phosphate/0.1 M NaCl/10 mM EDTA, pH 7.4). The cell suspension was frozen in liquid $N_2$ before storage at −80°. After thawing and rupture of the cells by sonication (Branson sonifier B-12, 70 W output, for 5 sec at 0°), 50 $\mu$L samples (160–230 $\mu$g of protein) were preincubated for 25 min at 37°. The enzyme reaction, which was performed in the presence of various drug concentrations (as indicated in the Results) for 40 min at 37°, was started by the addition of cofactors and substrate. The 100 $\mu$L assay mixture contained, besides the vastatins, 0.5 mM [$^{14}$C]HMG-CoA (sp. radioact. 3000–4500 dpm/nmol), 5 mM $NADP^+$, 50 mM glucose 6-phosphate, 0.7 unit of glucose-6-phosphate dehydrogenase, 50 mM potassium phosphate (pH 7.4), 50 mM NaCl, 10 mM EDTA, 5 mM dithiothreitol and 1.6–2.3 mg of Hep G2 cell protein/mL. The incubation was stopped by addition of 25 $\mu$L 1.2 M HCl, which contained 2–3 × $10^4$ dpm [$^3$H]mevalonic acid (New England Nuclear, sp. radioact. 5 Ci/mmol) as recovery standard.

Mevalonic acid was converted into the lactone form by incubation for 30 min at 37° and isolated by TLC on silica plates (Merck DC 60; developed in acetone/toluene = 1:1). Silica in between $R_f$-values 0.3 and 0.6 was scraped and $^3$H-/$^{14}$C-radioactivity counted. The [$^{14}$C]mevalonate formed was corrected for the recovery of [$^3$H]mevalonate (70–80%) and the HMG-CoA reductase activity was expressed in pmol of mevalonic acid formed/min/mg of cellular protein. The values are the averages of duplicate determinations and given as percentage of control.

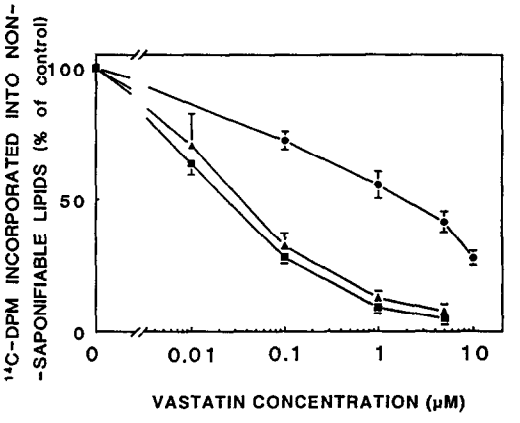


Fig. 1. Inhibition of sterol synthesis by vastatins in Hep G2 cells. Hep G2 cells were incubated with the indicated concentrations of either pravastatin (●), lovastatin (■) or simvastatin (▲) for 18 hr at 37° in the presence of [$^{14}$C]-acetate (0.4 $\mu$Ci/mL). The incorporation of label into non-saponifiable lipids was determined as described in Materials and Methods. Values are expressed as percentages of control (34,330 ± 4325 dpm/mg of cellular protein); means ± SEM (N = 4–5).

*Assay of squalene synthase activity.* The determination of squalene synthase activity in Hep G2 cell homogenates after incubation was performed as described previously [13]. Values, expressed as nmol of squalene formed/min/mg of cellular protein, were obtained from duplicate determinations in two homogenates of identically-treated cells. The average value for the individual homogenates agreed within 10%. Protein concentrations were determined according to Lowry *et al.* [20].

Values resulting from the experiments, which were separately performed at least three times (designated as N in the legends of the figures), are depicted as the mean percentages of the control values ± SEM. Statistical differences ($P < 0.05$) from control values, as determined by a paired *t*-test, are indicated in the figures.

## RESULTS

### *Inhibition of cholesterol synthesis by vastatins in Hep G2 cells*

Hep G2 cells were incubated with different concentrations of the drugs in the presence of [$^{14}$C]-acetate for 18 hr and the incorporation of $^{14}$C-label into non-saponifiable lipids was determined. As is shown in Fig. 1 simvastatin inhibited the cholesterol synthesis at lower concentrations ($IC_{50}$ = 34 nM than pravastatin ($IC_{50}$ = 1900 nM). The values for lovastatin were of the same order of magnitude as those for simvastatin ($IC_{50}$ = 24 nM).

### *Effect of vastatins on HMG-CoA reductase mRNA levels in Hep G2 cells*

In order to investigate the effect of the inhibitors

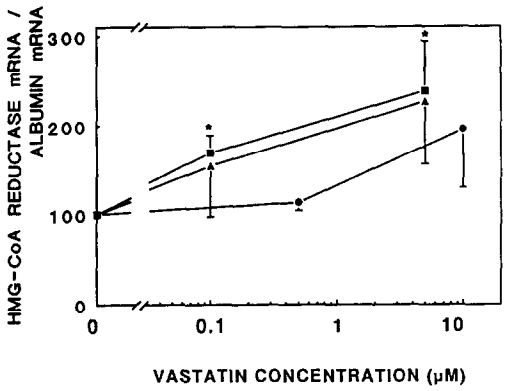


Fig. 2. Effect of vastatins on HMG-CoA reductase mRNA levels in Hep G2 cells. Cells were incubated for 18 hr at 37° with DMEM supplemented with 1% albumin and the indicated concentrations of either pravastatin (●), simvastatin (▲) or lovastatin (■). RNA was isolated from cells grown in two 10-cm$^2$ wells and the HMG-CoA reductase mRNA concentration was determined by northern blot hybridization as described in Materials and Methods. Values for the reductase mRNA levels, the ratio of the reductase- and the albumin-probe-derived blackening on the autoradiograph, are expressed as mean percentages of control ± SEM (N = 3). (*) Values are significantly different ($P < 0.05$) from control values using a paired *t*-test.

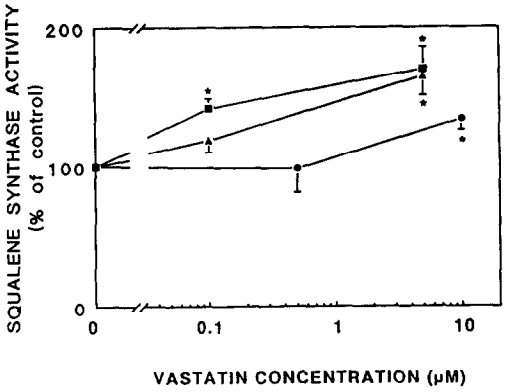


Fig. 3. Effect of vastatins on squalene synthase activity in Hep G2 cells. Cells were incubated with the different vastatins as described in the legend of Fig. 2. Squalene synthase activity was determined in the cell homogenate as described in Materials and Methods. Values are expressed as percentages of control (2.00 ± 0.10 nmol/min/mg of cellular protein); means ± SEM (N = 3). (*) Values are significantly different ($P < 0.05$) from control values.

on the feedback regulation of HMG-CoA reductase, after the 18 hr-incubation the HMG-CoA reductase mRNA levels were investigated by northern blot analysis. The levels of the albumin mRNA, which were not influenced by the reductase inhibitors, were used as an internal standard in the assay. The results are depicted in Fig. 2. The same concentration-dependent increase of the reductase mRNA levels by the simvastatin and lovastatin was observed, whereas only a smaller increase at a high pravastatin concentration was observed. These data correspond with the concentration-dependent inhibition of the cholesterol synthesis by the drugs (Fig. 1). The values obtained with lovastatin are in good agreement with the data published by Molowa and Cimis [21], using actin mRNA as an internal standard.

*Effect of vastatins on squalene synthase activity in Hep G2 cells*

Previously we have reported that in Hep G2 cells squalene synthase activity is regulated by sterols in the same way as the HMG-CoA reductase mRNA levels [13]. In order to extend those observations and to support the data depicted in Fig.2 the squalene synthase activity in the Hep G2 cell homogenates were determined after incubation with the different reductase inhibitors for 18 hr. As shown in Fig. 3, the squalene synthase activity was increased in the same way as was observed for the reductase mRNA.

*Inhibition of HMG-CoA reductase activity by vastatins directly in Hep G2 cell homogenates*

In order to exclude the possibility that the differences between the vastatins observed in intact

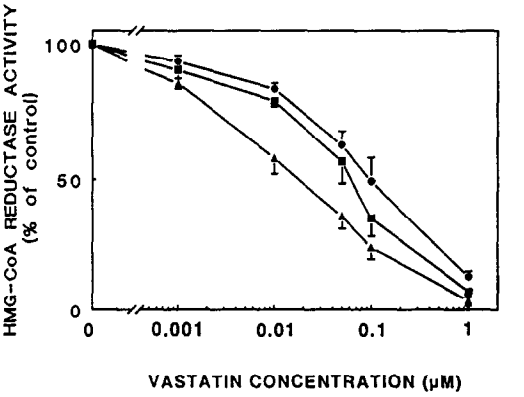


Fig. 4. Inhibition of HMG-CoA reductase activity in Hep G2 cell homogenate by vastatins. The HMG-CoA reductase assay was performed as described in Materials and Methods in 50 μL-samples of the same batch of Hep G2 cell homogenate in the absence or presence of the indicated concentrations of either pravastatin (●), lovastatin (■) or simvastatin (▲). The enzyme activity was expressed as percentage of controls (90.6 ± 10.4 pmol/min/mg of cellular protein) and depicted as the average value of three separately performed experiments ± SEM.

cells were the result of differences in the potency of the drugs to inhibit the Hep G2 HMG-CoA reductase, the inhibition of the enzyme activity was directly measured in the Hep G2 cell homogenate. Therefore, the HMG-CoA reductase assay was performed in Hep G2 cell homogenate in the presence of various concentrations of the three compounds. As can be seen in Fig. 4 simvastatin, lovastatin and pravastatin inhibited the enzyme to

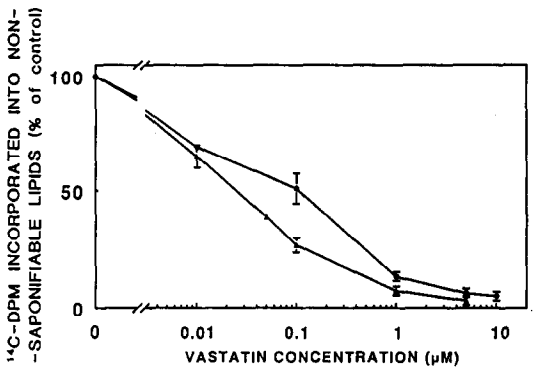


Fig. 5. Inhibition of sterol synthesis by vastatins in human hepatocytes. Human hepatocytes were incubated for 18 hr at 37° in Williams E medium/10% foetal bovine serum/135 nM insulin/50 nM dexamethasone with the indicated concentrations of either pravastatin (●) or simvastatin (▲). After 1 hr, 2 μCi/mL of [$^{14}$C]acetate was added and the incubation proceeded for 17 hr. The [$^{14}$C]non-saponifiable lipids have been determined as described in Materials and Methods. Values are expressed as percentages of control (28830 ± 6040 dpm/mg of cellular protein); means ± SEM (N = 3; for values with N < 3 no SEM has been given).

the same order of magnitude ($IC_{50}$ values of 18, 61 and 95 nM, respectively). The difference in inhibitory potency of maximal five times cannot explain the difference in inhibition of the cholesterol synthesis in intact cells (compare Fig. 1).

*Inhibition of cholesterol synthesis by vastatins in human hepatocytes*

As discussed below the results obtained with the Hep G2 cells can be explained by an impaired uptake of pravastatin by the cells, possibly by missing a specific hepatic transporter. In order to support this explanation the inhibition of the sterol synthesis in human hepatocytes in primary culture by simvastatin and pravastatin was investigated. The results are shown in Fig. 5. In contrast with the large difference in inhibitory potency of simvastatin and pravastatin in Hep G2 cells, in human hepatocytes pravastatin is much more potent and its $IC_{50}$-value (105 nM) was much closer to that of simvastatin (23 nM).

## DISCUSSION

The HMG-CoA reductase inhibitors simvastatin, lovastatin and pravastatin, presently in use as cholesterol-lowering drugs, inhibited the cholesterol synthesis in the human hepatoma cell line Hep G2 to a different extent. Under the conditions used, pravastatin was about 60–80 times less potent than simvastatin and lovastatin. This was also reflected in their influence on HMG-CoA reductase and squalene synthase mediated by the co-ordinate feedback regulation of these enzymes by inhibiting the synthesis of sterol suppressors. HMG-CoA reductase mRNA levels (Fig. 2) and squalene synthase activity (Fig. 3) were increased to the same extent by simvastatin and lovastatin and to a lesser extent by pravastatin.

The three drugs have comparable $IC_{50}$ values for the inhibition of HMG-CoA reductase activity in rat liver microsomal preparations (unpublished results; and Ref. 22) and inhibit the reductase activity at the same order of magnitude in Hep G2 cell homogenates (Fig. 4). Therefore, we explain the observed difference between the effects of simvastatin and lovastatin, on one hand, and of pravastatin, on the other, by limited transport of pravastatin through the Hep G2 cell membrane compared with the other two compounds. There is evidence [4, 7] that differences in uptake of the drugs play a role in the difference in inhibitory potency of pravastatin compared to lovastatin and simvastatin in hepatic cells versus non-hepatic cells. Very recently it was shown that the uptake of pravastatin in rat hepatocytes was carrier-mediated [23] and that a specific hepatic sodium-independent bile acid transporter was responsible for this action [24]. Hep G2 cells may have lost this transporter to a certain extent, limiting the uptake of pravastatin, while the two more lipophylic compounds are still able to enter the cells possibly by diffusion or via other transporters. This suggestion is supported by the observation that Hep G2 cells have lost the ability to transport taurocholate (Dr H. M. G. Princen, (personal communication) in contrast with freshly isolated human hepatocytes [25] and in which taurocholate and pravastatin are competitors for the same transporter [24]. Also the bilirubin transporter has been found to be strongly reduced in Hep G2 cells [26].

The presence of a transporter may possibly be influenced by the growth conditions of the Hep G2 cells, because we observed a different $IC_{50}$ value for pravastatin (18 hr-incubation 1900 nM) than was recently published by Nagata *et al.* [27] ($IC_{50}$ value pravastatin (18-hr incubation 1900 nM) than was recently published by Nagata *et al.* [27] ($IC_{50}$ value pravastatin 18-hr incubation > 10,000 nM) and by vastatins were of the same order of magnitude (24–80 nM) in these three independent studies.

Further support for a missing hepatic pravastatin-transporter in Hep G2 cells is given by our observation that in human hepatocytes in primary culture pravastatin is a much stronger inhibitor of cholesterol synthesis, comparable to simvastatin (Fig. 5). While the taurocholate transporter is present in these cells [25] this observation suggests that a specific transporter was involved in the action of pravastatin. Further investigations, using radiolabelled drugs, will give more insight into this mechanism.

From the results discussed above we conclude that the human hepatoma cell line Hep G2 is a good model for the study of the feedback regulation of the cholesterol synthesis. However, this cell line is less suitable as a human hepatocyte model for the study of specific uptake of drugs.

*Acknowledgements*—Mrs L. de Pagter is gratefully acknowledged for taking care of the Hep G2 culture. We thank Mrs M. Horsting for typing the manuscript.

## REFERENCES


1. Endo A, Kuroda M and Tanzawa K, Competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase by ML-236A and ML-236B fungal metabolites, having hypocholesterolemic activity. *FEBS Lett* **72**: 323–326, 1976.
2. Alberts AW, Chen J, Kuron G, Hunt V, Hoffman C, Rothrock J, Lopez M, Joshua H, Harris E, Patchett A, Monaghan R, Currie S, Stapley E, Albers-Shonberg G, Hensens O, Hirshfield J, Hoogstee K, Liesch J and Springer J, Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. *Proc Natl Acad Sci USA* **77**: 3957–3961, 1980.
3. Hoffman WF, Alberts AW, Anderson PS, Chen JS, Smith RL and Willard AK, 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors. 4. Side chain ester derivatives of mevinolin. *J Med Chem* **29**: 849–852, 1986.
4. Tsujita Y, Kuroda M, Shimada Y, Tanzawa K, Arai M, Kaneko I, Tanaka M, Masuda H, Tarumi C, Watanabe Y and Fujii S, CS-514, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase: tissue selective inhibition of sterol synthesis and hypolipidemic effect on various animal species. *Biochim Biophys Acta* **877**: 50–60, 1986.
5. Hunninghake DB, HMG-CoA reductase inhibitors. *Curr Opin Lipidol* **3**: 22–28, 1992.
6. Mosley ST, Kalinowski SS, Schafer BL and Tanaka RD, Tissue selective effects of inhibitors of 2-hydroxy-3-methylglutaryl coenzyme A reductase on cholesterol biosynthesis in lens. *J Lipid Res* **30**: 1411–1420, 1989.
7. Koga T, Shimada Y, Kuroda M, Tsujita Y, Hasegawa K and Yamazaki M, Tissue selective inhibition of cholesterol synthesis *in vivo* by pravastatin sodium, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor. *Biochim Biophys Acta* **1045**: 115–120, 1990.
8. Serajuddin ABT, Ranadive SA and Mahoney EM, Relative lipophilicities, solubilities, and stucture-pharmacological considerations of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors pravastatin, lovastatin, mevastatin and simvastatin. *J Pharm Sci* **80**: 830–834, 1991.
9. De Vries AM, Vermeer M, Bloemendal H and Cohen LH, Pravastatin and simvastatin differently inhibit cholesterol synthesis in human lens. *Invest Ophthalmol Vis Sci* **34**: 377–384, 1993.
10. Knowles BB, Howe CC and Aden DP, Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. *Science* **209**: 497–499, 1980.
11. Cohen LH, Griffioen M, Havekes L, Schouten D, Van Hinsbergh VWM and Kempen HJ, Effect of compactin, mevalonate and low-density lipoprotein on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and low-density-lipoprotein-receptor activity in the human hepatoma cell line Hep G2. *Biochem J* **222**: 35–39, 1984.
12. Cohen LH and Griffioen M, Regulation of 3-hydroxy-3-methylglutaryl-CoA reductase mRNA contents in human hepatoma cell line Hep G2 by distinct classes of mevalonate-derived metabolites. *Biochem J* **255**: 61–69, 1988.
13. Cohen LH, Van Miert E and Griffioen M, Regulation of squalene synthetase in human hepatoma cell line Hep G2 by sterols, and not by mevalonate-derived non-sterols. *Biochim Biophys Acta* **1002**: 69–73, 1989.
14. Boogaard A, Griffioen M and Cohen LH, Regulation of 3-hydroxy-3-methylglutaryl coenzyme. A reductase in human hepatoma cell line Hep G2; effect of inhibitors of cholesterol synthesis on enzyme activity. *Biochem J* **241**: 345–351, 1987.
15. Kooistra T, Bosma PJ, Töns HAM, Van den Berg AP, Meyer P and Princen HMG, Plasminogen activator inhibitor 1: biosynthesis and mRNA level are increased by insulin in cultured human hepatocytes. *Thromb Haemost* **62**: 723–728, 1989.
16. Bligh EG and Dyer WJ, A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**: 911–917, 1959.
17. Chomczynski P and Sacchi N, Single-step method of RNA isolation by acid guadinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162**: 156–159, 1987.
18. Church GM and Gilbert W, Genomic sequencing. *Proc Natl Acad Sci USA* **81**: 1991–1995, 1984.
19. Maniatis T, Fritch EF and Sambrook J, *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1982.
20. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
21. Molowa DT and Cimis GM, Coordinate regulation of low-density-lipoprotein receptor and 3-hydroxy-3-methylglutaryl-CoA reductase and synthase gene expression in Hep G2 cells. *Biochem J* **260**: 731–736, 1989.
22. Shaw MK, Newton RS, Sliskovic DR, Roth BD, Ferguson E and Krause BR, Hep G2 cells and primary rat hepatocytes differ in their response to inhibitors of HMG-CoA reductase. *Biochem Biophys Res Commun* **170**: 726–734, 1990.
23. Komai T, Shigehara E, Tokui T, Koga T, Ishigami M, Koroiwa C and Horoiuchi S, Carrier-mediated uptake of pravastatin by rat hepatocytes in primary culture. *Biochem Pharmacol* **43**: 667–670, 1992.
24. Ziegler K and Stünkel W, Tissue-selective action of pravastatin due to hepatocellular uptake via a sodium-independent bile acid transporter. *Biochim Biophys Acta* **1139**: 203–209, 1992.
25. Kwekkeboom J, Princen HMG, Van Voorthuizen EM, Meijer P and Kempen HJM, Comparison of taurocholate accumulation in cultured hepatocytes of pig, rat and man. *Biochem Biophys Res Commun* **162**: 619–625, 1989.
26. Stremmel W and Diede HE, Cellular uptake of conjugated bilirubin and sulfobromophthalein (BSP) by the human hepatoma cell line Hep G2 is mediated by a membrane BSP/bilirubin binding protein. *J Hepatol* **10**: 99–04, 1990.
27. Nagata Y, Hidaka Y, Ishida F and Kamei T, Effect of simvastatin (MK-733) on the regulation of cholesterol synthesis in Hep G2 cells. *Biochem Pharmacol* **40**: 843–850, 1990.