Effects of Simvastatin (MK-733) on Branched Pathway of Mevalonate

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Abstract—The effects of simvastatin (MK-733), a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, on the branched pathway of mevalonate metabolism were studied in Hep G2 cells. The synthesis of cholesterol, ubiquinone and dolichol were examined using various radiolabeled precursors. The effect on DNA synthesis was also determined. MK-733 at a concentration of 1 μM potently inhibited the incorporation of [3H]acetate into cholesterol (84%) without affecting that from [3H]mevalonolactone. Under these conditions, MK-733 reduced the incorporation of L-[14C]tyrosine into ubiquinone slightly (14%), although it did not suppress that from [3H]acetate. The incorporation of [3H]acetate into dolichol was slightly reduced by MK-733. On the contrary, the incorporation of [3H]-mevalonolactone into ubiquinone and dolichol was increased by MK-733. This apparent increase in incorporation was thought to be largely due to the higher specific radioactivity of the intracellular pool of mevalonate. The present study demonstrated that MK-733 slightly suppressed the synthesis of ubiquinone and dolichol in Hep G2 cells. However, the extent of their reduction was far less than the effect on cholesterol synthesis, suggesting that there were differences in substrate affinity between the enzymes participating in the cholesterol synthetic pathway and those in the ubiquinone or dolichol synthetic pathway. Furthermore, MK-733 did not affect DNA synthesis even at a concentration of 10 μM.

Mevalonate, which is formed by 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (EC 1.1.1.34), is a critical intermediate in the biosynthetic pathway of cholesterol in mammalian cells (1). The formation of mevalonate is regarded as the major regulatory step for de novo cholesterol biosynthesis (2). It is now recognized that several factors such as low density lipoprotein (LDL) cholesterol, oxygenated sterols and HMG-CoA reductase inhibitors, which influence the activity of HMG-CoA reductase and the rate of sterol synthesis in cultured cells, also influence the rate of ubiquinone (3–5) and dolichol (6) synthesis.

Simvastatin (MK-733) (7), a chemical derivative of lovastatin (MK-803, mevinolin) (8), is a prodrug of a potent competitive inhibitor of HMG-CoA reductase. The active β-hydroxy acid form of MK-733, L-645,969 (7), inhibited HMG-CoA reductase with an IC50 of 0.94 nM. It has been reported that both MK-803 and MK-733 in their lactone forms, in contrast to their corresponding β-hydroxy open acids, undergo preferential first pass sequestration by the liver (9, 10), which is the target organ for inhibition of HMG-CoA reductase. Hepatic uptake in the dog was approximately 60% for MK-803 and 90% for MK-733 (9). Recently Germershausen et al. reported that MK-803 and MK-733 (lactone forms) were more specific for the liver than pravastatin (open acid form of HMG-CoA reductase inhibitor) (11). It is considered that the lactone form of HMG-CoA reductase inhibitors readily undergo first-pass metabolism, hepatic sequestration and hydrolysis to the active form. Consequently, the plasma con-
centrations of MK-803 and MK-733 are lower than those of other HMG-CoA reductase inhibitors, causing it to be scarcely distributed among peripheral tissues in vivo (11). The effects of compactin and mevinolin on ubiquinone or dolichol synthesis have been studied using various extra-hepatic cell lines, non-mammalian cells and rat liver slices (3-5, 12-15). To evaluate the effect of MK-733 on the branched pathway of mevalonate metabolism in humans, human liver cells, which are the target sites, are considered to be more suitable than cells derived from extrahepatic tissues. The Hep G2 cell line has been reported to retain many human liver-specific functions and to secrete normal liver proteins, including apolipoproteins (16-19). The effects of MK-733 on the synthesis of cholesterol and other lipids such as free fatty acids, phospholipids and triacylglycerol in Hep G2 cells have been reported in a previous paper (20). It has been clearly demonstrated that MK-733 at a concentration of 20 μM inhibited cholesterol synthesis potently without affecting fatty acid, phospholipid and triacylglycerol synthesis. Therefore, we have chosen Hep G2 cell to examine the effects of MK-733 on the synthesis of ubiquinone and dolichol in a parallel comparison with cholesterol synthesis on the mevalonate pathway. Figure 1 shows the branched pathway of mevalonate metabolism in mammalian cells (3) and the inhibitory step of MK-733 in this pathway. Recently, Goldstein and Brown reported that non-sterol isoprenoids derived from mevalonate are vital for diverse cellular functions, ranging from cholesterol synthesis to growth control (21). Therefore, the effects of MK-733 on DNA synthesis were also investigated using [3H]-thymidine.

Materials and Methods

1. Materials: MK-733 (Lot No. L-644,128-000U026, purity 98.0%) was prepared in Merck Sharp and Dohme Research Laboratories (MSDRL, Rahway, NJ, U.S.A.). The chemical structure of MK-733 is shown in Fig. 2. MK-733 was dissolved in dimethylsulfoxide (DMSO). The final concentration of DMSO did not exceed 0.3%. [2-14C]Acetic acid, sodium salt (54.3 mCi/mmol) and L-[U-14C]tyrosine (486 mCi/mmol) were obtained from Amersham International plc. (Buckinghamshire, England). [3H]Acetic
acid, sodium salt (86.4 mCi/mmol), DL-[5-\(^{3}\)H] mevalonolactone (38.8 Ci/mmol) and [methyl-\(^{3}\)H]thymidine (2 Ci/mmol) were purchased from New England Nuclear (Boston, MA, U.S.A.). Ubiquinone (coenzyme Q10, from bovine heart), dolichol (C\(_{80}\)-C\(_{105}\), from porcine liver) and Q-cytosine arabinoside (Ara-C) were purchased from Sigma (St. Louis, MO, U.S.A.). All other chemicals used were of standard commercial high purity materials. Human lipoprotein-deficient serum (LPDS) was prepared as described by Brown et al. (22). Human low density lipoprotein (LDL, d: 1.020-1.063) was isolated from freshly prepared plasma by preparative ultracentrifugation (23).

2. Cell cultures: The established Hep G2 cell line, derived from human hepatoma, was obtained from American Type Culture Collection (Rockville, MD, U.S.A.). Cell stocks were grown in 80-cm\(^2\) flasks containing Eagle’s modified minimum essential medium (MEM, Flow Laboratories, McLean, VA, U.S.A.) supplemented with penicillin G (100 units/ml) and streptomycin (100 \(\mu\)g/ml) [medium A], with 10% (v/v) heat inactivated fetal bovine serum (FBS), and incubated in a humidified incubator (5% CO\(_2\)) at 37°C.

3. Incorporation into cholesterol, ubiquinone and dolichol of \([^{14}\text{C}]\)acetate, \([^{3}\text{H}]\)mevalonolactone or \([^{14}\text{C}]\)tyrosine: On day 0, 3x10\(^5\) cells/ml were seeded in 10 cm\(^2\) or 21 cm\(^2\) petri dishes containing medium A supplemented with penicillin G (100 units/ml) and streptomycin (100 \(\mu\)g/ml) [medium A], with 10% (v/v) heat inactivated fetal bovine serum (FBS), and incubated in a humidified incubator (5% CO\(_2\)) at 37°C.

Fig. 2. Chemical structure of simvastatin (MK-733).

and the medium was aspirated. The cells were washed with cold phosphate-buffered saline (PBS) three times. To determine the IC\(_{50}\) of MK-733 for cholesterol synthesis, the cells were dissolved in aqueous 15% KOH. The lipids in the cell lysate were saponified with 15% KOH in 59% ethanol for 1 hr at 75°C. After addition of water, the non-saponifiable lipids were extracted with petroleum ether twice, and the solvent was evaporated under a nitrogen stream. \([^{14}\text{C}]\)Cholesterol was isolated on a silica gel G plate (Art 5583, E. Merck, Darmstadt, West Germany) using hexane/diethyl ether/acetic acid (85:15:4, v/v) as a solvent system. For the determination of free cholesterol, ubiquinone or dolichol synthesis, the cells were harvested using a policeman, and disrupted by ultrasonication (Sonifier 185, Branson Sonic Power Co., Danbury, CT, U.S.A.). Lipids in the homogenate were extracted by the method of Folch et al. (24). Free cholesterol, ubiquinone and dolichol were separated by two-dimensional thin-layer chromatography (TLC) (Art 5554, E. Merck) according to the method of Sexton et al. (25). Ubiquinone (coenzyme Q10) and dolichol (C\(_{80}\)-C\(_{105}\)) were used as markers. Authentic standards on TLC were visualized by exposure to I\(_2\) vapor. The bands corresponding to authentic cholesterol, ubiquinone and dolichol were scraped into a toluene based liquid scintillation cocktail. The radioactivity was counted by a liquid scintillation counter (TRI-CARB 2000CA, Packard Instrument Co., IL, U.S.A.). The efficiency of the combined extraction and TLC was determined to be 89±1.7%. This value was confirmed in a separate experiment using radio-labeled cholesterol. The distribution of radioactivity on the TLC plate was also examined by autoradiography by exposing it to X-ray film (Fuji X-ray film, Fuji Photo Film Co., Ltd., Tokyo, Japan).

4. Incorporation of \([^{3}\text{H}]\)thymidine into acid-insoluble fraction: Hep G2 cells grown in a 10 cm\(^2\) dish were treated with MK-733 for 3 hr, and then 1 \(\mu\)Ci of \([^{3}\text{H}]\)thymidine was added to the medium. After incubation at 37°C for 1 hr, the medium was removed and the cells were washed with PBS three times. Incorporation of \([^{3}\text{H}]\)thymidine into the trichloroacetic acid-insoluble fraction of the
cells was determined according to the method of Kaneko et al. (26). The radioactivity in the acid-insoluble fraction was determined as described above.

5. Determination of protein: The protein concentrations were determined according to the method of Lowry et al. (27) using bovine serum albumin as a standard.

6. Statistical analysis: Data from these studies were statistically analyzed using the Mann-Whitney U-test (28). The variations in all mean values are expressed as the standard deviation (S.D.).

Results

1. Cholesterol synthesis: The rate of incorporation of [14C]acetate or [3H]mevalonolactone into cholesterol in Hep G2 cells was linear at least up to 18 hr (data not shown). Hep G2 cells were preincubated for 1 hr with various concentrations of MK-733, and then one of the radiolabeled precursors was added to the culture medium and incubated for 2 hr. Cholesterol was synthesized at a rate of 3.3 nmol/hr/mg cell protein in control. As shown in Fig. 3, MK-733 inhibited the incorporation of [14C]acetate into cholesterol and the concentration required for 50% inhibition was approximately 20 nM. On the other hand, the incorporation of [3H]mevalonolactone was not affected by MK-733 at concentrations up to 3 μM. MK-733 dose-dependently inhibited cholesterol synthesis from [14C]acetate without affecting that from [3H]mevalonate. These results indicated that MK-733 is a specific inhibitor of HMG-CoA reductase in Hep G2 cells.

Fig. 3. Effects of MK-733 on the incorporation of [14C]acetate or [3H]mevalonolactone into cholesterol in Hep G2 cells. Hep G2 cells cultured for 7 days in 1 ml of medium were preincubated with the indicated concentrations of MK-733 for 1 hr and then either 1 μCi/ml of [14C]acetate (○) or 2 μCi/ml of [3H]mevalonolactone (▼) at the final concentration of 1 mM or 50 μM was added to the medium. After 2 hr incubation, the radioactivity in cholesterol was quantified as described in Materials and Methods. Each value represents the mean of duplicate determinations. Observed values in the control (100%) were 11000 dpm/2 hr/dish and 4000 dpm/2 hr/dish when [14C]acetate or [3H]mevalonolactone was used.

2. Ubiquinone and dolichol synthesis: In human fibroblasts, ubiquinone 10 has been reported to be the predominant ubiquinone (3-4). However, ubiquinone synthesis in Hep G2 cells has not been demonstrated yet. Therefore, we examined ubiquinone and dolichol synthesis from [14C]acetate. Figure 4 shows the radiolabeled spots synthesized...
Table 1. Effects of MK-733 and LDL on the incorporation of \(^{3}H\)acetate or \(^{14}C\)tyrosine into ubiquinone and on the incorporation of \(^{3}H\)acetate into dolichol and cholesterol in Hep G2 cells

| Addition to medium | Incorporation of labeled precursors (dpm/mg protein/18 hr) | Ubiquinone | Dolichol | Cholesterol |
|-------------------|----------------------------------------------------------|------------|----------|-------------|
|                   |                                                          | \([^{3}H]\)acetate | \([^{14}C]\)tyrosine | \([^{3}H]\)acetate | \([^{3}H]\)acetate |
| Control           |                                                          | 1870±81 (100)  | 150±15 (100)  | 826±115 (100)  | 419000±17500 (100) |
| LDL               | 100 \(\mu g/ml\)                                       | 1750±66 (94)   | 136±20 (91)   | 739±43 (89)    | 177000±6870* (42) |
| MK-733            | 0.01 \(\mu M\)                                         | 2110±197* (113)| 150±13 (100)  | 886±101 (107)  | 340000±18100* (81) |
| MK-733            | 0.1 \(\mu M\)                                          | 2250±128* (120)| 144±14 (96)   | 896±48 (84)    | 194000±16000* (46) |
| MK-733            | 1 \(\mu M\)                                            | 2220±147* (119)| 129±13 (88)   | 483±110* (58)  | 68300±3280* (16) |
| MK-733 + LDL      | 1 \(\mu M\)                                            | 1380±54* (74)  | 96±9* (64)    | 306±46* (37)   | 36400±1390* (9)   |
| MK-733 + unlabeled MEV | 1 \(\mu M\)                                   | N.T.   | 145±1 (97)   | N.T. |
|                   | 1 \(mM\)                                                | N.T.     | N.T.       | N.T. |

Hep G2 cells cultured for 7 days in 3 ml of medium were preincubated with each substance indicated in the table for 1 hr. Then 14.5 \(\mu Ci/ml\) of \(^{3}H\)acetate (final conc.: 3 mM) and 0.6 \(\mu Ci/ml\) of \([^{14}C]\)tyrosine (final conc.: 181 \(\mu M\)) were added to the medium (3 ml). After 18 hr incubation, ubiquinone, dolichol and cholesterol were extracted, and the radioactivity in each fraction was quantified as described in Materials and Methods. Each value represents the mean±S.D. of triplicate determinations. The value in parenthesis is the relative % of each control. Significantly different from the value in each control: *\(P<0.05\). N.T.: Not tested. Observed values in the control are shown from the left side to the right on the table as follows: 1700, 140, 730 and 370000 (dpm/18 hr/dish).
from [14C]acetate on two-dimensional TLC visualized by autoradiography. [14C]Acetate was converted to not only the end-product of the mevalonate pathway but also to free fatty acids, triacylglycerol and phospholipid. Radiolabeled spots on TLC co-migrated well with the authentic standards of ubiquinone 10 or dolichol (C80-105) and were well-separated from other lipids. This showed that ubiquinone 10 was a major product among the ubiquinones in Hep G2 cells.

To investigate the effects of MK-733 on ubiquinone and dolichol synthesis in Hep G2 cells, [3H]acetate was used as a precursor for the synthesis of the isoprenoid chain of ubiquinone and dolichol. LDL has been reported to reduce ubiquinone synthesis in cultured cells (4), because it reduced HMG-CoA reductase activity. Therefore, LDL was used as a reference. Table 1 shows the effects of MK-733 and LDL on the incorporation of [3H]acetate into cholesterol, ubiquinone and dolichol. MK-733, at a concentration of 1 μM, inhibited cholesterol synthesis potently, while it did not affect ubiquinone synthesis. A high concentration of MK-733 (1 μM) inhibited dolichol synthesis by 42%, while it inhibited cholesterol synthesis by 84%. LDL inhibited cholesterol synthesis by 58%, and it reduced ubiquinone and dolichol synthesis by less than 10%. The combination of MK-733 and LDL additively inhibited the synthesis of cholesterol, ubiquinone and dolichol. In the incorporation from [3H]acetate, isotope dilution is considered to affect the results through the preincubation period (1 hr). To exclude the effect of isotope dilution, Maltese and Aprille recommended the use of [14C]tyrosine as a precursor for ubiquinone synthesis (5). Therefore, [14C]tyrosine was also used as a precursor for the synthesis of the benzoquinone ring of ubiquinone (Table 1). Ubiquinone synthesis from [14C]tyrosine was reduced by 100 ng/ml of LDL, 1 μM MK-733 or LDL plus MK-733. Unlabeled mevalonolactone (1 mM) suppressed the inhibition of ubiquinone synthesis by MK-733. These results show that MK-733 suppressed ubiquinone and dolichol synthesis slightly, but the extent of their reduction was far less than the effect on cholesterol synthesis. It has been reported that compactin, an HMG-CoA reductase inhibitor, increases the incorporation of [3H]-mevalonate into [3H]ubiquinone (4). In Hep G2 cells, similar results were obtained (Table 2). MK-733 increased the incorporation of [3H]mevalonolactone into ubiquinone and dolichol dose-dependently. LDL also increased the incorporation into them. MK-733 and LDL additively increased the incorporation of [3H]mevalonolactone into ubiquinone and dolichol.

3. DNA synthesis: In order to determine whether MK-733 affects DNA synthesis, the

| Addition to medium | Incorporation of [3H]mevalonolactone (dpm/mg protein/18 hr) |
|-------------------|----------------------------------------------------------|
|                   | Ubiquinone | Dolichol |
| Control           | 7350 (100) | 2630 (100) |
| LDL 100 μg/ml     | 13500 (184) | 4340 (165) |
| MK-733 0.3 μM     | 21800 (297) | 4810 (183) |
| MK-733 1 μM       | 34300 (467) | 5630 (214) |
| MK-733 3 μM       | 53800 (732) | 6950 (260) |
| MK-733 1 μM + LDL | 65700 (894) | 10900 (414) |

Hep G2 cells cultured for 7 days in 3 ml of medium were preincubated with each substance indicated in the table for 1 hr. Then 10 μCi/ml of [3H]mevalonolactone (final conc.: 3 μM) was added to the medium. After 18 hr incubation, ubiquinone and dolichol were extracted, and the radioactivity in each fraction was quantified as described in Materials and Methods. Each value represents the mean of duplicate determinations. The value in parenthesis is the relative % of each control. Values of the control are 7100 dpm/18 hr/dish in ubiquinone 10 and 2500 dpm/18 hr/dish in dolichol.
effect on the incorporation of \( ^{3}H \) thymidine into the trichloroacetic acid-insoluble fraction was examined. MK-733 was found to have no effect on DNA synthesis measured by the incorporation of \( ^{3}H \) thymidine into the trichloroacetic acid-insoluble fraction, even at 10 \( \mu \)M where sterol synthesis from \( ^{14}C \) acetate was almost completely blocked (Table 3). On the other hand, Ara-C markedly suppressed the incorporation of \( ^{3}H \) thymidine into the acid-insoluble fraction. During the incubation period, 10 \( \mu \)M MK-733 and Ara-C did not have any cytotoxic effects in Hep G2 cells morphologically.

### Table 3. Effects of MK-733 on the incorporation of \( ^{3}H \) thymidine into the trichloroacetic acid-insoluble fraction in Hep G2 cells

| Addition to medium | Incorporation of \( ^{3}H \) thymidine (dpm \( \times 10^{-3} / \text{mg protein/hr} \) |
|--------------------|--------------------------------------------------|
| Control            | 1019±36 (100)                                    |
| MK-733 0.1 \( \mu \)M | 1011±47 (99)                                     |
| KM-733 1 \( \mu \)M   | 985±9 (97)                                       |
| KM-733 10 \( \mu \)M  | 977±11 (96)                                      |
| Ara-C 1 \( \mu \)M    | 68±4* (7)                                        |

Hep G2 cells cultured for 3 days in 1 ml of medium were preincubated with MK-733 or \( \beta \)-cytosine arabinoside (Ara-C) for 3 hr. Then, 1 \( \mu \)Ci/ml of \( ^{3}H \) thymidine (carrier free) was added to the medium. After 1 hr incubation, the radioactivity in the acid-insoluble fraction was quantified as described in Materials and Methods. Each value represents the mean±S.D. of triplicate determinations except the control (n=5). The value in parenthesis is the relative % of each control. Significantly different from the value in the control: *P<0.05. Value of the control was 47000 dpm/hr/dish.

### Discussion

It has been reported that mevalonate is converted to various substances including three known end-products: cholesterol, the polyisoprene side chain of ubiquinone (coenzyme Q) and dolichol in eucaryotic cells (3, 6, 29) in the branched pathway of mevalonate metabolism. In the present study, the effects of MK-733 on the branched pathway of mevalonate including the synthesis of cholesterol, ubiquinone and dolichol were examined in Hep G2 cells. Siperstein reported that isoprenoid derived from mevalonate plays an essential role in the growth of all eucaryotic cells and HMG-CoA reductase might have an acute function in DNA replication (30).

Therefore, we also examined the effect of MK-733 on DNA synthesis. The effect of HMG-CoA reductase inhibitors, such as compactin and MK-803, on ubiquinone or dolichol synthesis have been reported using extra-hepatic cells or liver slices (3-5, 15). However, the effects of HMG-CoA reductase inhibitor on the synthesis of cholesterol, ubiquinone and dolichol have not been compared in mammalian cell lines at the same time. MK-733 has been shown to have greater tissue selectivity towards the liver in vivo (9, 11). Therefore, in the present study, Hep G2 cells, which are known to have characteristics similar to human liver cells, were used to predict the effect of MK-733 on the mevalonate pathway in human liver. The synthetic rate of cholesterol from \( ^{14}C \) acetate in Hep G2 cells (3.3 nmol/hr/mg cell protein) was found to be approximately 10 times higher than that in human fibroblasts (0.3 nmol/hr/mg cell protein) as reported (22). MK-733 inhibited cholesterol synthesis from \( ^{14}C \) acetate dose-dependently without affecting that from \( ^{3}H \) mevalonate (Fig. 3). The IC50 value of MK-733 in the incorporation of \( ^{14}C \) acetate into cholesterol was about 20 nM (20). The radiolabeled spots synthesized from \( ^{14}C \) acetate on two-dimensional TLC showed that ubiquinone 10 was a major product among ubiquinones in Hep G2 cells (Fig. 4). MK-733 at 1 \( \mu \)M did not inhibit ubiquinone synthesis from \( ^{3}H \) acetate while it inhibited cholesterol synthesis by 84% (Table 1). To avoid the possibility of isotope dilution, \( ^{14}C \) tyrosine was used as a precursor for the synthesis of the benzoquinone ring of ubiquinone. MK-733 at a con-
centration of 1 μM slightly inhibited the incorporation of [14C]tyrosine into ubiquinone (14%). These results suggest that ubiquinone synthesis is reduced by the lack of an isoprenoid chain supply from mevalonate, because MK-733 decreased mevalonate production by inhibiting HMG-CoA reductase. This was confirmed by an experiment showing that the addition of unlabeled mevalonolactone suppressed the inhibition of ubiquinone synthesis from [14C]tyrosine by MK-733. However, it seems that the synthetic rate of ubiquinone is little influenced by MK-733 in Hep G2 cells. Maltese et al. demonstrated that a high concentration of lovastatin (mevinolin: 25 μM) inhibited ubiquinone synthesis from [14C]tyrosine in cultured neuroblastoma cells (5).

MK-733 at a concentration of 1 μM inhibited the incorporation of [3H]acetate into dolichol by 42%, while it inhibited the incorporation into cholesterol by 84% (Table 1). Keller investigated the effect of lovastatin on dolichylphosphate synthesis from [3H]-acetate. He showed that lovastatin inhibited dolichylphosphate synthesis in the same manner as that of cholesterol in rat liver slices (15).

LDL reduced ubiquinone and dolichol synthesis from radiolabeled precursors by less than 10% (Table 1). Faust et al. (31) reported that both HMG-CoA reductase and squalene synthetase activity in human fibroblasts were suppressed by the addition of LDL. Under these conditions, they suggested that the isoprene pool size, such as the amount of farnesyl pyrophosphate, remains constant and the amount of end product derived from isoprenoid would not change dramatically. Our data agree with their observations. Both MK-733 and LDL increased the incorporation of [3H]mevalonolactone into ubiquinone and dolichol dose-dependently (Table 2). The increased incorporation of radioactivity was thought to be caused by the higher specific radioactivity of mevalonate in the intracellular pool, which had resulted from the lack of endogenous mevalonate to dilute the radiolabeled precursor. In human fibroblasts, Faust et al. found that the rate of ubiquinone 10 synthesis from [3H]mevalonate was enhanced under the suppressive conditions of cholesterolgenesis by LDL and compactin (3). However, Nambudiri et al. clearly proved that the increased synthesis of ubiquinone 10 from [3H]mevalonate by LDL and compactin was due to a higher specific radioactivity of the intracellular pool of mevalonate that resulted from the lack of endogenous mevalonate to dilute the [3H]mevalonate, using 4[U-14C]hydroxybenzoic acid (HB) as a marker (4). They concluded that the level of HMG CoA reductase plays a role in determining the rate of ubiquinone synthesis. We found that MK-733 also increases the incorporation of [3H]mevalonate into dolichol. It is suggested that the isoprene pool used for the synthesis of ubiquinone and dolichol may be identical.

Recently, Goldstein and Brown stated in their review article that non-sterol isoprenoid derived from mevalonate is related to the entrance into the DNA synthesis phase and cell cycle concerning cell growth (21). Therefore, the effects of MK-733 on DNA synthesis were examined. MK-733 did not affect DNA synthesis even at a concentration of 10 μM. On the other hand, ara-C, a specific DNA polymerase inhibitor, markedly inhibited it. In our experiment, MK-733 also had no effect on the synthesis of macromolecules like DNA, RNA and protein in L-cells (data not shown) as described with compactin (26) and with pravastatin (32). The present study demonstrated that MK-733 slightly suppressed the synthesis of ubiquinone and dolichol in Hep G2 cells; however, the extent of their reduction was far less than the effect on cholesterol synthesis. Nambudiri et al. reported that the addition of LDL decreased the incorporation of [14C]acetate into ubiquinone by 35%, while it decreased cholesterol synthesis by 90% in human fibroblasts (3). James and Kandutsch reported that 25-hydroxycholesterol inhibited dolichol and sterol synthesis from [14C]-acetate in a concentration-dependent manner, but it inhibited sterol synthesis more potently than dolichol synthesis (6). Our results agree with their observations. Enzymes that participate in ubiquinone and dolichol synthesis may have higher affinity to their substrates than the enzymes in the cholesterol synthetic pathway. It is thought that MK-
733 scarcely affects dolichol and ubiquinone synthesis in human liver.

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