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Schneiders, M.S.

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Manipulation of isoprenoid biosynthesis as possible therapeutic option in mevalonate kinase deficiency

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Manipulation of Isoprenoid Biosynthesis as a Possible Therapeutic Option in Mevalonate Kinase Deficiency

Marit Schneiders, Sander M. Houten, Marjolein Turkenburg, Ronald J. A. Wanders, and Hans R. Waterham

Laboratory Genetic Metabolic Diseases, Departments of Clinical Chemistry and Pediatrics, Emma Children’s Hospital, Academic Medical Center, University of Amsterdam

Summary

Objective. In cells from patients with the autoinflammatory disorder mevalonate kinase (MK) deficiency, which includes the hyperimmunoglobulin D with periodic fever syndrome, MK becomes the rate-limiting enzyme in the isoprenoid biosynthesis pathway. This suggests that up-regulation of residual MK activity in these patients could be a way in which to prevent or alleviate the associated symptoms. We studied the effect of 2 specific inhibitors of isoprenoid biosynthetic enzymes on the residual activity of MK in cells from patients with MK deficiency. Methods. Skin fibroblasts from MK-deficient patients and from controls were cultured for 7 days with either simvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, or zaragozic acid A, an inhibitor of squalene synthase. Following culture, MK activity, MK protein levels, MVK messenger RNA levels, and the effect on the pathway flux toward non-sterol isoprenoid biosynthesis were determined. Results. Treatment of the fibroblasts with either of the inhibitors led to a marked increase in residual MK enzyme activity, which was largely attributable to increased MVK gene transcription. This effect was even more pronounced when the cells were cultured in lipoprotein-depleted medium. The flux toward non-sterol isoprenoid end-product synthesis was reduced when cells were treated with simvastatin but was partly restored by concomitant treatment with zaragozic acid A. Conclusion. Our results indicate that manipulations of the isoprenoid biosynthesis pathway that promote the synthesis of non-sterol isoprenoids may provide an interesting therapeutic option for the treatment of MK deficiency.

Mevalonate kinase (MK) deficiency is an autosomal-recessive inborn error of metabolism. Patients with MK deficiency present with characteristic autoinflammatory symptoms, including recurring episodes of high fever associated with headache, arthritis, nausea, abdominal pain, diarrhea, and skin rash (1,2). Originally, 2 distinct clinical entities associated with this defect were defined: classic mevalonic aciduria (3) and hyperimmunoglobulin D with periodic fever syndrome (HIDS) (1). However, it is now clear that those 2 entities represent the severe and mild ends, respectively, of the
clinical and biochemical spectrum of MK deficiency. Indeed, patients with the HIDS presentation typically have recurrent episodes of fever with associated symptoms (1), whereas patients with the mevalonic aciduria presentation show, in addition to these episodes, developmental delay, dysmorphic features, ataxia, cerebellar atrophy, and psychomotor retardation; patients with mevalonic aciduria may die during early childhood (2).

This difference in clinical presentation can be explained by the fact that at the biochemical level, cells from patients with the HIDS presentation still show residual MK enzyme activity that is 1–8% of the activity in cells from healthy controls (4-7). In contrast, enzyme activity in cells from patients with the mevalonic aciduria presentation is below the level of detection (2). This difference in residual enzyme activity is also reflected by high levels of mevalonic acid in plasma and urine from patients with the mevalonic aciduria presentation (2) and low to moderate levels of mevalonic acid in plasma and urine from patients with the HIDS presentation (5).

\[
\text{HMG-CoA reductase} \rightarrow \text{Simvastatin} \\
\downarrow \\
\text{Mevalonate kinase} \\
\downarrow \\
\text{Phosphomevalonate kinase} \\
\downarrow \\
\text{Geranylgeranyl pyrophosphate synthase} \\
\downarrow \\
\text{Squalene synthase} \quad \text{ZAA} \\
\downarrow \\
\text{Cholesterol} \\
\downarrow \\
\text{RhoA, Rac1}
\]

**Figure 1. The isoprenoid biosynthesis pathway.**

HMG-CoA = 3-hydroxy-3-methylglutaryl coenzyme A; SREBPs = sterol regulatory element binding proteins; ZAA = zaragozic acid A.
MK catalyzes the phosphorylation of mevalonate to produce 5-phosphomevalonate and is the next enzyme in the isoprenoid biosynthesis pathway after 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Fig. 1), which, under normal conditions, is the rate-limiting enzyme in this pathway (8). The isoprenoid biosynthesis pathway provides cells with a variety of essential bioactive molecules, including sterols and non-sterol compounds that have pivotal functions in multiple cellular processes, ranging from cell growth and differentiation to protein glycosylation and numerous signal transduction pathways.

To prevent a shortage of end products or overaccumulation of intermediates, the flux through the isoprenoid biosynthesis pathway is tightly regulated by the levels of its end products (8). HMG-CoA reductase plays a central role in this regulation and is subject to different regulatory mechanisms. For example, the efficiency of HMG-CoA reductase messenger RNA (mRNA) translation is dictated by the requirement of the cell for non-sterol isoprenoids, whereas the degradation rate of HMG-CoA reductase protein is dependent on the requirement for both sterol and non-sterol isoprenoids (8). At the transcription level, the HMG-CoA reductase gene and all other genes encoding enzymes of the isoprenoid biosynthesis pathway are subject to transcriptional feedback regulation coordinated by the so-called sterol regulatory element binding proteins (SREBPs), in particular SREBP type 2 (9-11). SREBPs are conditional positive transcription factors that enhance gene transcription in the absence of sterols but are not required for basal transcription when sterols are present (9,10).

Previously, we postulated that the episodes of fever in MK-deficient patients are attributable to a temporary shortage of 1 or more of the non-sterol isoprenoids that are required for down-regulation of the inflammatory response precipitated by fairly harmless events such as physical stress or vaccination (12-14). This lack of down-regulation can be explained by the fact that in patients with MK deficiency, MK has become the rate-limiting enzyme in the isoprenoid biosynthesis pathway instead of HMG-CoA reductase. Under normal conditions, the flux through this pathway in MK-deficient patients will be sufficiently high for most cellular processes requiring isoprenoids (13,14). However, any small increase in body temperature will result in a rapid decrease in residual MK activity, due to the deleterious, temperature-sensitive effect on MK protein maturation and stability of most mutations found in these patients, which will lead to a rather instant disturbance of the flux through the pathway and, as a consequence, a shortage of end products (14).

Based on this postulate, we investigated the effect of 2 specific inhibitors of isoprenoid biosynthetic enzymes on the residual activity of MK in cells from MK-deficient patients. For these studies, we used cultured primary skin fibroblasts from such patients, in which the isoprenoid biosynthesis pathway can be readily manipulated by varying the culturing conditions, as reported previously (13,14). Our results show that both simvastatin, an inhibitor of HMG-CoA reductase, and zaragozic acid A (ZAA), an inhibitor of squalene synthase, give rise to increased MK activities, which is
attributable to enhanced transcription of the gene MVK. This increase in MK activity renders the flux through the pathway less dependent on the conversion of mevalonate to phosphomevalonate. Our in vitro results suggest that treatment of patients with inhibitors of isoprenoid biosynthesis may provide a therapeutic option that could lead to less frequent and/or shorter episodes of fever.

PATIENTS AND METHODS

Cell culture
Primary fibroblast cell lines obtained from 2 MK-deficient patients with the HIDS presentation, i.e., HIDS1 (MVK alleles H2OP/V377I) and HIDS2 (MVK alleles I268T/V377I), 1 patient with the mevalonic aciduria presentation, i.e., MA (MVK alleles I268T/I268T), and a healthy control subject were cultured in a nutrient mixture of Ham’s F-10 medium with L-glutamine and 25 mM HEPES (Invitrogen, Breda, The Netherlands) supplemented with 10% fetal calf serum (FCS) (Invitrogen) or 10% lipoprotein-depleted FCS (Biowest, Nuaille’, France), as indicated. Cells were grown in T-162 culture flasks (Costar, Corning, NY) until confluency, after which the cells received fresh culture medium supplemented with either simvastatin (a gift from Merck, Sharpe, and Dohme BV, Haarlem, The Netherlands) or ZAA (a gift from Merck, Rahway, NJ) at the indicated concentrations or with only the solvents used to solubilize these drugs, followed by further incubation for 7 days. Incubation with only the solvents did not result in a noticeable effect on MK activity when compared with cells cultured in the absence of solvents (data not shown). Cells were then harvested by trypsinization, washed once with phosphate buffered saline and twice with 0.9% NaCl, divided into 2 pellets, snap-frozen in liquid nitrogen, and stored at -80°C until analyzed further.

Simvastatin was prepared as a 10-mM stock solution as described previously (13). ZAA was prepared as a 50-mM stock solution by dissolving the drug in DMSO (Merck, Darmstadt, Germany).

MK activity
MK activity was measured as described previously (15), using 14C-labeled mevalonate (PerkinElmer Life Sciences, Boston, MA) as a substrate.

Immunoblot analysis
Equal amounts of protein were separated by sodium dodecyl (lauryl) sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose by semidy blotting. Affinity-purified antibodies directed against human MK (16) were used at a 1:500 dilution. Antigen-antibody complexes were visualized with swine anti-rabbit horseradish peroxidase conjugate (Dako, Glostrup, Denmark), using the enhanced chemiluminescence system (Amersham Biosciences, Little Chalfont, UK). To verify equal transfer of proteins, each blot was reversibly stained with ponceau S prior to
incubation with antibodies. Densitometric analysis of immunoblots was performed using Advanced Image Data Analyzer (AIDA) software (Raytest, Straubenhardt, Germany).

Quantitative real-time reverse transcription–polymerase chain reaction (RT-PCR) analysis
The expression levels of MVK mRNA were related to the expression levels of GAPDH mRNA, using the LightCycler System (Roche, Mannheim, Germany). To this end, total RNA was isolated from primary skin fibroblasts using TRIzol (Invitrogen, Carlsbad, CA) extraction, after which complementary DNA (cDNA) was prepared using a first-strand cDNA synthesis kit for RT-PCR (Roche). The MVK fragment was amplified using the following primer set: 5’-CTCCGATACCATCAAGGG-3’ (forward) and 5’-GCTCACACTCCAGGGAGA-3’ (reverse). The GAPDH fragment was amplified using the following primer set: 5’-ACCACCATGGAGAAGGCTGC-3’ (forward) and 5’-CTCAGTGCCCAGGATGC-3’ (reverse). Three independent experiments were performed, and in every sample the expression of MVK and GAPDH was determined in duplicate. Data were analyzed using LightCycler software, version 3.5 (Roche) and the program LinRegPCR, version 7.5 (17) for analysis of real-time PCR data.

Membrane and cytosol separation
Primary fibroblast cells from the patient with mevalonic aciduria were cultured in triplicate in a nutrient mixture containing Ham’s F-10 with L-glutamine and 25 mM HEPES (Invitrogen, Breda, The Netherlands) supplemented with 10% FCS (Invitrogen, Breda, The Netherlands) in T-75 culture flasks (Costar) until confluency. One day prior to treatment, all cells received fresh medium. On day zero, simvastatin was added to the culture medium (2 sets of flasks) at a final concentration of 0.2 µM. After 1 day, one set of flasks received ZAA at a final concentration of 50 µM. One day later, all cells were harvested by trypsinization and used directly for membrane and cytosol separation, as described previously (13). Membrane fractions were separated by SDS-PAGE and immunoblotted with RhoA monoclonal antibody (sc-418) diluted 1:1,000 (Santa Cruz Biotechnology, Santa Cruz, CA) or Rac1 monoclonal antibody (1:10,000 dilution) (Upstate Biotechnology, Lake Placid, NY).

Statistical analysis
Statistical analysis was performed using one-way analysis of variance followed by Dunnett’s 2-sided t-test or a 2-tailed paired t-test where appropriate. P values less than 0.05 were considered significant.

RESULTS
Up-regulation of MK activity by simvastatin
Primary fibroblast cells from the patients with HIDS, the patient with mevalonic aciduria, and healthy subjects were incubated for 7 days with simvastatin, an inhibitor of HMG-
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CoA reductase. The concentrations of simvastatin used for this study were based on our previously published results, which showed that cells from patients with mevalonic aciduria are more sensitive to simvastatin than are cells from patients with HIDS, due to the difference in residual MK activity (13).

Pilot experiments showed that effects were clearly visible after 4–7 days of incubation (data not shown). The incubations were performed in standard medium supplemented with FCS (FCS+) and in medium supplemented with lipoprotein-depleted FCS (FCS-), in order to remove an excess of sterols. Measurement of MK activity in cells cultured in FCS+ medium without simvastatin revealed low activity in the HIDS cells (1–2% of the activity observed in control cells) and activity in the mevalonic aciduria cells that was below the level of detection (Fig. 2A). Incubation of the cells in FCS+ medium supplemented with simvastatin resulted in increased MK activities in both the HIDS cells (6–10-fold) and the control cells (2-fold). Incubation of the cells in FCS- medium also resulted in elevated MK activity in the HIDS cells (5–9-fold) and
Figure 2.
A, Specific enzyme activities (pmol/mg*minute) and immunoblot analysis (15 µg of protein/lane) of mevalonate kinase (MK) in fibroblast lysates from 1 control, 2 hyper-immunoglobulin D and periodic fever syndrome (HIDS), and 1 mevalonic aciduria (MA) cell line. The control and HIDS cell lines were incubated in the presence or absence of 1 µM simvastatin (sim) for 7 days. The mevalonic aciduria cell line was incubated in the presence or absence of 200 nM simvastatin for 7 days. Cells were cultured in medium containing lipoprotein-rich fetal calf serum (FCS+) or medium containing lipoprotein-depleted FCS (FCS-), as indicated. B, MK activity in the HIDS and mevalonic aciduria cell lines expressed as the percentage of the activity measured in the control cell line cultured under the same conditions. C, Relative mRNA expression of MVK and GAPDH in control, HIDS, and mevalonic aciduria cell lines, presented as 1,000 × the MVK-to-GAPDH ratio. D, MK activity as the ratio of the relative MVK mRNA levels in control, HIDS, and mevalonic aciduria cell lines. Numbers above the bars indicate the increase in these ratios when compared with the same cells cultured in standard FCS+ medium. Results are the mean and SD of 3 independent experiments. Statistical analysis was performed using one-way analysis of variance followed by Dunnett’s t-test. NA = not applicable. * = P < 0.05; ** = P < 0.005 versus standard medium.
the control cells (2-fold) when compared with cells incubated in FCS+ medium. The highest MK activity was observed in the cells incubated in FCS- medium supplemented with simvastatin. Compared with the standard (FCS+) medium, incubation in FCS-medium supplemented with simvastatin resulted in 28–42-fold increased MK activity in cells from patients with HIDS, 4.5-fold increased activity in control cells, and even a marked increase in cells from the patient with mevalonic aciduria, although activity in the latter cells remained very low (Fig. 2A).

When the MK activity in MK-deficient cell lines was compared with the activity measured in control cells cultured under the same conditions, it appeared that the relative increase in MK activity due to simvastatin and/or lipoprotein-depleted medium in the MK-deficient cells was higher than that in control cells (Fig. 2B).

Immunoblot analysis of lysates prepared from the cells, using specific antibodies against human MK, showed that the increased MK activity was paralleled by increased levels of MK protein in the cells (Fig. 2A). This increased MK activity can be explained only in part by enhanced transcription of the MVK gene (Fig. 2C), because, when compared with the increase in MVK mRNA levels, the increase in MK activity in the HIDS cells was markedly higher than that in control cells (Fig. 2D).

**Up-regulation of MK activity by ZAA**

The fact that simvastatin is an inhibitor of HMG-CoA reductase, which catalyzes an early step in isoprenoid synthesis, suggests that treatment with simvastatin would (in principle) inhibit the synthesis of both sterol and non-sterol isoprenoids. Because results of our previous studies (12,13) indicated that the shortage underlying the symptoms of MK deficiency is at the level of non-sterol isoprenoids, we also studied the effect of ZAA on MK activity in cells. ZAA is a specific inhibitor of squalene synthase, the first enzyme committed exclusively to sterol isoprenoid biosynthesis, and thus will inhibit only the synthesis of sterol isoprenoids and redirect the flux toward synthesis of non-sterol isoprenoids. The concentration of ZAA used in this study was based on dose-response studies in which we tested ZAA concentrations ranging from 5 µM to 100 µM and determined MK activity after 7 days of incubation (data not shown).

Similar to simvastatin treatment, treatment with ZAA resulted in increased MK enzyme activity in cells incubated in standard (FCS+) medium (1.5–4-fold) (Fig. 3A). In lipoprotein-depleted medium (FCS-), supplementation with ZAA resulted in 35–48-fold increased MK activity in cells from patients with HIDS, 5-fold increased activity in control cells, as well as a marked increase in activity in cells from the patient with mevalonic aciduria. In addition, the relative increase in MK activity in MK-deficient cell lines incubated in FCS- medium supplemented with ZAA was markedly higher than that in control cells cultured under the same conditions (Fig. 3B).

Immunoblot analysis of lysates prepared from the cells confirmed that the increased activities were again correlated with the MK protein levels (Fig. 3A). Quantification of the MVK mRNA levels showed that the increased MK activities were partly correlated with enhanced MVK gene transcription, under lipoprotein-depleted culture conditions.
and particularly in the presence of ZAA (Fig. 3C). As with simvastatin, however, when compared with the increase of MVK mRNA levels, the increase in MK activity in the HIDS cells was markedly higher than that in control cells (Fig. 3D).

**A**

![Graph showing MK activity](image)

**B**

![Graph showing % of MK activity versus control](image)

**C**

![Graph showing MVK/GAPDH mRNA ratio](image)
Up-regulation of MK by manipulation of isoprenoid biosynthesis

Figure 3.
A, Specific enzyme activities (pmol/mg*minute) and immunoblot analysis (15 µg of protein/ lane) of MK in fibroblast lysates from 1 control, 2 HIDS, and 1 mevalonic aciduria cell line after 7 days of incubation in the presence or absence of 50 µM zaragozic acid A (ZAA). Cells were cultured in FCS+ medium or FCS- medium, as indicated. B, MK activity in the HIDS and mevalonic aciduria cell lines expressed as the percentage of the activity measured in the control cell line cultured under the same conditions. C, Relative mRNA expression of MVK in control, HIDS, and mevalonic aciduria cell lines, presented as 1,000 x the MVK-to-GAPDH ratio. D, MK activity as the ratio of the relative MVK mRNA level in control, HIDS, and mevalonic aciduria cell lines. Numbers above the bars indicate the increase in these ratios when compared with the same cells cultured in standard FCS+ medium. Results are the mean and SD of 3 independent experiments. Statistical analysis was performed using one-way analysis of variance followed by Dunnett’s t-test. * = P < 0.05; ** = P < 0.005 versus standard medium. See Figure 2 for other definitions.

Flux studies in cells treated with simvastatin and ZAA
Because residual MK activity in MK-deficient cells becomes increased upon treatment with simvastatin as well as ZAA, we also examined whether the flux through the isoprenoid biosynthesis pathway is increased under these conditions. We used the geranylgeranylation status of 2 small GTPases, RhoA and Rac1, as a marker for the flux toward non-sterol isoprenoid biosynthesis. These small GTPases require covalent binding of the non-sterol isoprenoid geranylgeranyl to tether them to the membrane. Our previous studies indicated that despite the MK deficiency, fibroblasts from MK-deficient patients have normal levels of geranylgeranylated RhoA in their membranes (13), from which we concluded that under normal growth conditions the cells have sufficient flux through the pathway to support normal synthesis of geranylgeranylated proteins. Those studies also indicated, however, that cells from patients with HIDS and those from patients with mevalonic aciduria display higher sensitivity to treatment with
simvastatin, with decreased levels of membrane-bound RhoA observed with relatively low concentrations of simvastatin (13).

Consistent with our previous findings, we again observed decreased levels of membrane-bound RhoA and Rac1 when we treated cells from the patient with mevalonic aciduria with simvastatin, indicating a decreased pathway flux (Fig. 4). Subsequent treatment of the simvastatin-treated cells with ZAA re-established the flux toward non-sterol isoprenoid biosynthesis, as shown by the reappearance of RhoA and Rac1 protein in the membranes to levels nearly similar to those in untreated cells (Fig. 4).

**DISCUSSION**

In this study, we sought to determine whether it is possible to up-regulate residual MK activity in cells from MK-deficient patients by manipulating the isoprenoid biosynthesis pathway. The rationale for this study is based on our earlier observations, which indicated that in MK-deficient patients, MK has become the rate-limiting enzyme in isoprenoid biosynthesis that determines the flux through the pathway (14,18). Thus, in principle, up-regulation of residual MK activity in patients would shift the rate-limiting step back to HMG-CoA reductase and make the pathway flux less dependent on MK activity. As a consequence, patients would be less susceptible to external precipitating events that negatively influence MK activity, which affects the flux through the pathway and the production of certain end products.

We studied the effect of 2 specific inhibitors of the isoprenoid biosynthesis pathway, simvastatin and ZAA. Simvastatin is a widely used competitive inhibitor of HMG-CoA reductase, the enzyme preceding MK in the pathway. ZAA is a competitive inhibitor of squalene synthase (19), the first enzyme dedicated exclusively to the production of sterol isoprenoids. Treatment of fibroblasts from MK-deficient patients with either of the inhibitors led to a marked increase in residual MK enzyme activity, mainly due to increased \( \text{MVK} \) gene transcription. This effect was already evident in cells cultured in standard (lipoprotein-rich) medium but was further enhanced when the cells were cultured in lipoprotein-depleted medium.

These findings are consistent with the known feedback regulation of isoprenoid biosynthesis genes by SREBPs, which become activated under these conditions, because the production of sterol end products will further decrease due to the inhibitors. Remarkably, however, after incubation with simvastatin and ZAA in lipoprotein-depleted medium, the relative increase in MK activity in cells from patients with HIDS and those from the patient with mevalonic aciduria was much higher than that in control cells cultured under the same conditions. This cannot be explained by increased \( \text{MVK} \) transcription alone, as was evident from the increase in MK activity over \( \text{MVK} \) mRNA produced in these cells. This suggests that inhibition of isoprenoid biosynthesis somehow promotes the formation of stable mutant MK protein.
Because our previous studies indicated that the shortage in MK-deficient patients involves primarily, if not exclusively, non-sterol isoprenoids including geranylgeranyl groups (12, 13), we also studied the effect of the 2 inhibitors on the flux toward non-sterol isoprenoid biosynthesis. This was done by determining the presence of the small GTPases RhoA and Rac1 in cell membranes. Both proteins are synthesized as soluble proteins and require a covalently bound geranylgeranyl moiety to become localized in the cellular membrane, where they exert their effect. Our results showed that although treatment with simvastatin led to up-regulation of MK, it apparently had a negative effect on the overall flux toward geranylgeranyl synthesis at this particular concentration, because both RhoA and Rac1 became less abundant in the cellular membrane.

Thus, whereas treatment with simvastatin seemed promising with regard to increasing MK activity, the consequence of inhibiting the preceding enzyme HMG-CoA reductase appears to be negative for the flux. This finding indicates that one needs to be cautious when treating MK-deficient patients with simvastatin, because the balance between inhibiting HMG-CoA reductase and inducing MK activity may be critical, especially in MK-deficient patients in whom the pathway flux is very sensitive to external influences. This was also suggested by the negative outcome of treatment of 2
patients with mevalonic aciduria using lovastatin, a drug that is similar to simvastatin (2). This treatment provoked severe clinical crises in those patients. However, it was recently reported that treatment of 6 HIDS patients with simvastatin (20) did not provoke clinical crises in those patients, in contrast to the patients with mevalonic aciduria, which is a more severe disease. Although no statistical difference was observed with respect to the severity, frequency, and occurrence of febrile attacks, a clear decrease in the total number of febrile days was observed. These findings in patients with mevalonic aciduria and in patients with HIDS are consistent with our previous observations that, in vitro, cells from patients with mevalonic aciduria appear more sensitive to simvastatin compared with cells from patients with HIDS or those from control subjects (13).

When compared with treatment with simvastatin, treatment with ZAA or other squalene synthase inhibitors seems more promising. Not only does ZAA treatment result in up-regulation of MK activity, it also leads to an increased flux toward synthesis of geranylgeranyl groups. This was particularly evident when cells were incubated with both simvastatin and ZAA, which shows that the negative effect on the flux by simvastatin is counterbalanced by ZAA, leading to a reappearance of RhoA and Rac1 in the cellular membrane. Treatment of cells with ZAA in the absence of simvastatin led to minimally increased levels of RhoA and Rac1 protein in the membranes (data not shown).

Being a specific squalene synthase inhibitor, ZAA was previously reported to be a potential drug for the treatment of hypercholesterolemia (19). However, ZAA has not been developed further for this indication, nor are there any reports of this drug being tested in humans; the only reported treatments with ZAA were performed in animals (19,21-25). In fact, of all reported squalene synthase inhibitors, only BMS-188494 (the prodrug of BMS-187745) was tested in healthy human volunteers, which led to changes in urinary farnesyl pyrophosphate metabolite (dioic acid) excretion when doses of >100 mg were given for 4 weeks, without apparent negative effects (26,27). This drug is no longer available, however.

Although in recent years the insights into the pathogenesis underlying the symptoms of mevalonic aciduria and HIDS have increased (18), no efficacious treatment for MK-deficient patients is currently available. In individual HIDS cases, clinical improvement through treatment with corticosteroids (28), etanercept (29,30), or leukotriene inhibitors (28) has been reported, but in the majority of patients these treatments did not have beneficial effects, and they have not led to a general treatment of MK deficiency. Our in vitro results suggest that treatment of patients with inhibitors of isoprenoid biosynthesis may provide a possible therapeutic option to alleviate or even prevent the episodes of inflammation. It should be noted here that besides using inhibitors of isoprenoid biosynthesis, culturing cells in lipoprotein-depleted medium also led to the up-regulation of MK activity in cells from patients with HIDS. This finding suggests that patients may also benefit from a cholesterol-depleted diet.
References


