Polyisoprenoid Epoxides Stimulate the Biosynthesis of Coenzyme Q and Inhibit Cholesterol Synthesis*

Magnus Bentinger†, Michael Tekle§, Kerstin Brismar¶, Tadeusz Chojnacki†, Ewa Swiezewska‡, and Gustav Dallner†§

From the †Department of Biochemistry and Biophysics, Stockholm University, 10691 Stockholm, Sweden, ‡Rolf Luft Research Centre for Diabetes and Endocrinology, Karolinska Institutet, 17176 Stockholm, Sweden, and §Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 02-106 Warsaw, Poland

In our search for compounds that up-regulate the biosynthesis of coenzyme Q (CoQ), we discovered that irradiation of CoQ with ultraviolet light results in the formation of a number of compounds that influence the synthesis of mevalonate pathway lipids by HepG2 cells. Among the compounds that potently stimulated CoQ synthesis while inhibiting cholesterol synthesis, derivatives of CoQ containing 1–4 epoxide moieties in their polyisoprenoid side chains were identified. Subsequently, chemical epoxidation of all-trans-polyisoprenols of different lengths revealed that the shorter farnesol and geranylgeraniol derivatives were without effect, whereas the longer derivatives of solanesol enhanced CoQ and markedly reduced cholesterol biosynthesis. In contrast, none of the modified trans-trans-poly-cis-polyisoprenols exerted noticeable effects. Tocotrienol epoxides were especially potent in our system; those with one epoxide moiety in the side-chain generally up-regulated CoQ biosynthesis by 200–300%, whereas those with two such moieties also decreased cholesterol synthesis by 50–90%. Prolonged treatment of HepG2 cells with tocotrienol epoxides for 26 days elevated their content of CoQ by 30%. In addition, the levels of lipids by HepG2 cells. Among the compounds that potently stimulated CoQ synthesis while inhibiting cholesterol synthesis, derivatives of CoQ containing 1–4 epoxide moieties in their polyisoprenoid side chains were identified. Subsequently, chemical epoxidation of all-trans-polyisoprenols of different lengths revealed that the shorter farnesol and geranylgeraniol derivatives were without effect, whereas the longer derivatives of solanesol enhanced CoQ and markedly reduced cholesterol biosynthesis. In contrast, none of the modified trans-trans-poly-cis-polyisoprenols exerted noticeable effects. Tocotrienol epoxides were especially potent in our system; those with one epoxide moiety in the side-chain generally up-regulated CoQ biosynthesis by 200–300%, whereas those with two such moieties also decreased cholesterol synthesis by 50–90%. Prolonged treatment of HepG2 cells with tocotrienol epoxides for 26 days elevated their content of CoQ by 30%. In addition, the levels of mRNA encoding enzymes involved in CoQ biosynthesis were also elevated by the tocotrienol epoxides. The site of inhibition of cholesterol synthesis was shown to be oxidosqualene cyclase. In conclusion, epoxide derivatives of certain all-trans-polyisoprenoids cause pronounced stimulation of CoQ synthesis and, in some cases, simultaneous reduction of cholesterol biosynthesis by HepG2 cells.

Coenzyme Q (CoQ)2 is present in the membranes of all animal cells, where it performs a number of essential functions. In addition to the roles it plays in the mitochondrial respiratory chain and as the only lipid-soluble antioxidant synthesized endogenously, CoQ also participates in extramitochondrial electron transport, functional modification of mitochondrial uncoupling proteins, regulation of the mitochondrial permeability transition pore, and modulation of the levels of certain receptors on the surface of blood monocytes (1, 2). Furthermore, CoQ influences the expression of a large number of genes whose products are involved in a number of metabolic processes (3, 4).

Under a variety of conditions, the tissue level of CoQ is reduced, an alteration that is considered to exert a negative impact of cellular functions. During aging, the levels of this lipid in all organs of the rat and human are severely decreased (5). Moreover, diseases such as cardiomyopathy, muscle degeneration, and cancer of the liver are associated with significantly lower tissue levels of CoQ (6). In addition, genetic disorders characterized by impaired biosynthesis of CoQ involve serious metabolic disturbances that can be partially counteracted by dietary supplementation (7, 8). Under such conditions, elevating tissue levels of CoQ would be beneficial.

The biosynthesis of cholesterol is influenced by a number of endogenous metabolites, including oxysterols, squalene oxide, farnesol, and its derivatives, geranylgeraniol, prenyl phosphates, and sterols, that activate meiosis (9). It appears highly probable that as yet unidentified metabolites also regulate CoQ biosynthesis. For instance, in connection with diseases in which oxidative stress is a contributing factor, such as Alzheimer and prion diseases, precarcinogenic conditions, and diabetes, biosynthesis of CoQ is up-regulated (10).

The relatively short half-life of CoQ in tissues, varying between 50 and 125 h, leads to the formation of significant amounts of metabolites, some of which may be regulators of the biosynthesis of this lipid (11). Degradation products isolated from the urine and feces of rats and guinea pigs contain an intact and fully substituted ring together with a short, carboxylated side chain. These compounds are subject to glucuronidation or phosphorylation prior to excretion into the bile and urine, respectively (12, 13). Moreover, various breakdown products of CoQ may be produced in association with UV irradiation and lipid peroxidation. Thus, UV irradiation of skin rapidly reduces its content of CoQ, whereas such irradiation of crystals of this lipid yields a number of degradation products (14, 15). In its reduced form, CoQ in submitochondrial particles and liposomes is protected from lipid peroxidation, but the oxidized form is subject to extensive breakdown to more polar, as yet unidentified compounds (16).

Inhibition of cholesterol synthesis is of considerable medical interest, and, at present, inhibitors of 3-hydroxy-3-methylglu-
Isopteryl-CoA reductase, the statins, are employed almost exclusively for this purpose. One drawback of statin treatment is that it also interferes with CoQ synthesis and protein isoprenylation (17, 18). Therefore, effective inhibition of cholesterol synthesis downstream from the branch point represented by farnesyl-PP synthase might prove to be highly beneficial.

In the present investigation, compounds containing an epoxidated side chain, obtained by UV irradiation of CoQ, are shown to exert profound effects on the biosynthesis of CoQ and cholesterol. Furthermore, all-trans-polysoprenes epoxidated chemically influence the biosynthesis of lipid products of the mevalonate pathway in tissue culture. By varying the number of epoxide moieties on the polysoprene chain, differential effects on CoQ and cholesterol synthesis can be achieved.

**EXPERIMENTAL PROCEDURES**

**Reagents**—α-Unsaturated polyisoprenols were prepared from Sorbus suecica, as described previously (19). Dolichol-11 was prepared by saturation of the α-terminal isoprene residue of plant undecaprenol (20). Dolichol-18 was isolated from human liver (21). (R,S)-5-[3H]Mevalonolactone was synthesized employing [3H]sodium borohydride (15 Ci/mmol; GE Healthcare) as described by Keller (22). [3H]lanosterol was synthesized from [3H]mevalonate according to Downs et al. (23). Tocotrienols were purchased from Davos Life Sciences Pte. Ltd. (Singapore). All other chemicals were procured from Sigma.

For purposes of epoxidation, the various lipids were dissolved in dichloromethane and then mixed with 3-chloroperoxybenzonic acid (77%) dissolved in dichloromethane to obtain a 1:2 molar ratio of lipid to 3-chloroperoxybenzonic acid (24). Following incubation at room temperature for 30 min, the solvent was removed by evaporation under nitrogen, and the residue was redissolved in hexane/diethyl ether (4:1). Subsequently, the individual epoxides were separated by column chromatography on silica gel 60 (230–400 mesh) utilizing a gradient from hexane to an equal mixture of hexane and diethyl ether for elution. The individual fractions thus obtained were purified further on a RP-18 column (LiChroprep 40–63 μm; Merck) with methanol/acetonitrile (3:1) as the eluent.

Solanesyl-PP was prepared according to Poppak et al. (25). The polyisoprenes were acetylated by treatment with acetic anhydride/pyridine (1:1) at room temperature for 1 h, and the resulting products were isolated by silica chromatography. Phthalidimation of solanesol was performed according to Sen and Roach (26).

**UV Irradiation and Separation of Products**—To obtain compounds formed in response to UV irradiation, CoQ10 dissolved in ethanol/water (9:1) (0.4 mg/ml) was irradiated at 190–300 nm, 500 microwatts/cm² for 30 min. Following evaporation of the solvents, the residue was dissolved in hexane and applied to a silica gel column, which was then eluted with a gradient starting from 100% hexane and finishing with hexane/diethyl ether (1:2).

**Cell Cultures**—Human hepatoblastoma (HePG2) cells were cultured in 10 ml of Dulbecco’s modified Eagle’s medium (Invitrogen) containing 1 g of glucose/100 ml, 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 mg/ml). When 70% confluence was reached, the medium was changed, and the compounds to be investigated were added in 50–100 μl of dimethyl sulfoxide to give a final concentration of 2 μM, or, in the case of tocotrienols, 5 μM. Twenty-four hours later, 0.5 mCi of [3H]mevalonate (3.25 Ci/mmol) was added, and incubation continued for an additional 8 h. When the substrate was [3H]lanosterol (0.28 Ci/mmol), the amount added was 5 μCi. Finally, the cells were harvested by trypsinization and stored at −20 °C for later analysis.

**Extraction and Chromatography of Lipids**—Lipids were extracted from cells with chloroform/methanol/water (1:1:0.3) at 37 °C for 1 h with magnetic stirring (27). The extracts thus obtained were adjusted to achieve a final chloroform/methanol/water ratio of 3:2:1, and complete phase separation was then accomplished by centrifugation. The lower chloroform phase was removed and evaporated to dryness under a flow of nitrogen, and the residue was subsequently redissolved in chloroform. After evaporation of the solvents, the neutral lipids were dissolved in chloroform/methanol (2:1).

For analysis of cholesterol, cholesterol metabolites, squalene, and CoQ, the samples were subjected to reversed-phase HPLC using a Supelcosil LC-18 column (3 μm, 4.0 × 75 mm) equipped with an LC-18 Supelguard (Supelco) column (28). For separation, a stepwise gradient involving methanol/water (9:1) in pump system A and methanol/2-propanol/hexane (2:1:1) in pump system B was employed at a flow rate of 1.1 ml/min. The gradient began at 15% solvent B, with increases to 47% after 8 min, 67% after 16 min, and 100% after 24 min. The lipids in the eluent were monitored at 210 and 275 nm with a UV detector, and labeling of radioactive products was measured with an online detector of radioactivity. Ergosterol and CoQ₉ were used as internal standards.

For the analysis of individual products following irradiation or chemical epoxidation of CoQ, this same HPLC procedure was utilized. However, HPLC analysis of tocotrienol epoxides was performed with a linear gradient starting from methanol/water at a ratio of 7:3 and ending with methanol/water at a ratio of 9:1. The flow rate was 1.1 ml/min, and the program time was 25 min.

Following chemical epoxidation, the products were separated by silica gel column chromatography as described above. Subsequently, the individual fractions were analyzed on silica gel-60 plates (Merck) and HPTLC RP-18 plates (Merck), and the spots were visualized with iodine. In the case of CoQ, solanesol, squalene, vitamin K₂, and polyisoprenes 7 and 11, the solvent system used for development was benzene/ethyl acetate (9:1) for the silica gel-60 plates and acetone/methanol (2:1) for HPTLC RP-18 plates. For farnesol and geranylgeraniol, the corresponding solvent systems were benzene/ethyl acetate (4:1) and acetone/methanol (1:1), whereas for dolichols, benzene/ethyl acetate (9:1) and 100% acetonitrile were employed, respectively. Finally, benzene/ethyl acetate (6:1) and acetone/methanol/water (6:3:1) were utilized for the separation of tocotrienols.
Mass Spectrometry and Determination of Protein—Negative ion ES spectra were recorded on a triple quadrupole mass spectrometer, as described earlier (13). Protein was determined with the bicinchoninic acid kit (Sigma).

Real Time PCR—Quantitative real time PCR was employed to access levels of gene expression. For this purpose, total RNA was isolated from HepG2 cells using the Qiagen Mini RNeasy plus minikit, and 100 ng of this RNA was reverse-transcribed into cDNA using random hexamer oligonucleotides and TaqMan reverse transcription reagents in a final volume of 20 μl. This cDNA was then mixed with SYBR Green PCR master mix and appropriate primers, and PCR analysis was performed with an ABI Prism 7300 sequence detector (Applied Biosystems). The genes analyzed and the respective primers are shown in Table 1.

Statistical Analyses—The results obtained are expressed as means ± S.D., and the differences between two groups were evaluated for statistical significance by Student’s t test. Differences between several groups were analyzed by analysis of variance, followed by Dunnett’s test. p values below 0.05 were considered statistically significant.

RESULTS

Effects of UV Light on CoQ10—Irradiation of a solution of CoQ10 and subsequent evaporation of the solvent and separation by TLC chromatography revealed a large number of products that were more hydrophilic than the parent lipid as well as some more hydrophobic compounds. Consequently, in order to obtain fractions for more detailed analysis, the irradiated mixture was subjected to column chromatography on silica gel employing elution with a solvent mixture of increasing hydrophilicity. When aliquots of the fractions were added to cultures of HepG2 cells (a cell line derived from a human liver cancer that contains primarily CoQ with a decaprenyl side chain), six of the fractions obtained resulted in a reduced rate of incorporation of [3H]mevalonate into cholesterol (Fig. 1A). At the same time, CoQ synthesis was enhanced to various extents (Fig. 1B). As illustrated for fraction 28 (Fig. 1C), fractions collected from the silica gel column were found to contain heterogeneous and complex mixtures of compounds upon analysis by reversed-phase HPLC.

Effects of Chemically Epoxidated CoQ and Solanesol—Certain peaks in the HPLC chromatograms exhibited elution times identical to derivatives of CoQ containing 1–4 epoxide moieties (prepared by treating CoQ10 with 3-chloroperoxybenzoic acid) and could be separated efficiently by column chromatography on silica gel, followed by chromatography on C18 HPTLC plates (Fig. 2A). Mass spectrometric analysis confirmed that the molecular masses of the compounds thus obtained were those of CoQ10 plus 16, 32, 48, and 64 daltons, respectively. Furthermore, although the side chain contained one or
Isoprenoid Epoxides Modulate CoQ and Cholesterol Synthesis

more epoxide moieties, spectroscopic investigation demonstrated that the quinone ring had not been modified. Previous studies have also concluded that various epoxidated derivatives are produced by UV irradiation of CoQ (14).

The biological effects of chemically epoxidated CoQ10 on HepG2 cells were examined. Cholesterol synthesis by HepG2 cells was not influenced by the presence of derivatives of CoQ with one or two epoxide moieties in the culture medium but was potently reduced by derivatives containing three or four such moieties (Fig. 2B). In contrast, biosynthesis of CoQ was enhanced by all four types of derivatives. Since our analyses showed that only the polyisoprenoid side chain is modified in these derivatives, we also tested the effects of the corresponding epoxides of solanesol, the side chain of CoQ. Solanesol containing 1–4 epoxide moieties elevated CoQ synthesis while practically eliminating cholesterol synthesis.

Effects of Epoxidated Derivatives of Various Poly-cis- and All-trans-polyisoprenoids—Poly-cis-α-saturated polyprenols constitute the majority of isoprenoid lipids that occur naturally, particularly in plants, whereas in animal tissues, α-saturated dolichols are predominant. Two α-unsaturated polyrenols (7 and 11 isoprene units) and two α-unsaturated polyrenols (11 and 18 isoprene units) were epoxidated, and these derivatives were found to exert only minor effects on the biosynthesis of cholesterol and CoQ by HepG2 cells (Table 2). Epoxidated derivatives of the two short all-trans-lipids farnesol and geranlygeranyl (which were also acetylated in order to enhance their uptake into the cells) did not alter cholesterol or CoQ synthesis significantly. Epoxidated vitamin K2 inhibited cholesterol synthesis by 33% and elevated CoQ synthesis by 27%. Epoxidated squalene (possessing one or two epoxide moieties in the middle portion of the isoprenoid chain, in contrast to the natural substrate for cholesterol biosynthesis, 2,3-oxidosqualene) proved to be a potent inhibitor of cholesterol synthesis (70% reduction). Epoxidated derivatives of solanesol and its phosphorylated, acetylated, and phthalimidated forms produced virtually total inhibition of cholesterol synthesis and, at the same time, stimulated CoQ synthesis by ~40%. All of the epoxidated isoprenoids were tested at a level of 2 μM, a concentration at which none of the parent nonepoxidated compounds exerted any influence on cholesterol or CoQ synthesis (not shown).

Effects of Epoxy Tocotrienols—Tocopherols contain a phytol-type side chain, whereas the side chain of tocotrienols consists of three unsaturated isoprenoid residues. Four types of tocotrienols, with differing numbers and distributions of methyl groups on the chromanol ring, occur naturally. Employing the same procedure for epoxidation as described above, tocotrienols possessing one or two epoxide moieties were produced and subsequently isolated by chromatography.

| TABLE 2 | Biosynthesis of cholesterol and CoQ by HepG2 cells in the presence of various polyisoprenoid epoxides |
| --- | --- |
| **Polyisoprenoid epoxides** | **Percentage of control** |
| **Cholesterol** | **Coenzyme Q10** |
| Control | 100 ± 4 | 100 ± 6 |
| **Poly-cis** | | |
| Polyrenol 7-cis | 92 ± 4 | 114 ± 9 |
| Polyrenol 11-cis | 92 ± 5 | 106 ± 5 |
| Dolichol-11 | 93 ± 6 | 113 ± 10 |
| Dolichol-18 | 96 ± 3 | 114 ± 5 |
| **All-trans** | | |
| Farnesol | 101 ± 3 | 100 ± 4 |
| Farnesyl-acetate | 103 ± 4 | 105 ± 8 |
| Geranylgeraniol | 102 ± 5 | 107 ± 11 |
| Geranylgeranyl-acetate | 102 ± 4 | 109 ± 12 |
| Vitamin K2 | 67 ± 2* | 127 ± 1* |
| Squalene | 30 ± 5* | 111 ± 8 |
| Solanesol | 3 ± 1* | 138 ± 14* |
| Solanesyl-phosphate | 7 ± 2* | 136 ± 13* |
| Solanesyl-acetate | 4 ± 2* | 145 ± 18* |
| Solanesyl-phthalimide | 3 ± 1* | 142 ± 17* |

a *p < 0.0001.  
* *p < 0.005.
To various and moderate extents, the parent compounds enhanced the rate of this process, whereas all of the derivatives containing one or two epoxide moieties stimulated CoQ biosynthesis 2.5–3.5-fold. Thus, certain of the tocotrienol epoxides selectively elevated CoQ synthesis, whereas others also reduced the rate of cholesterol biosynthesis by HepG2 cells.

In order to investigate the toxicity of tocotrienol epoxides in our cultures, HepG2 cells were cultured in the presence of various tocotrienol epoxides at concentrations (60 μM) 12-fold higher than those routinely employed here. The morphology, growth, and lipid metabolism of the cells were not altered under these conditions, indicating a very low degree of toxicity.

Effects of Prolonged Treatment—In the experiments described above, rates of biosynthesis were determined by monitoring incorporation of [3H]mevalonate. The potential physiological impact is, however, dependent on the changes in the actual amounts of CoQ. Therefore, HepG2 cells were cultured for 26 days in the presence of monoeoxyp-γ-tocotrienol (which stimulates CoQ biosynthesis), during which period the cellular lipid composition was analyzed (Fig. 4). The cellular content of CoQ increased continuously, reaching a level after 26 days of treatment that was 30% higher than in untreated, control cultures. At the same time, the cellular content of cholesterol was not altered, as expected, since γ-tocotrienol with only one epoxide moiety does not influence the biosynthesis of this steroid by HepG2 cells.

Effects on mRNA Levels—In order to elucidate the mechanism underlying the effects of these various epoxide derivatives on CoQ biosynthesis by HepG2 cells, the levels of mRNA encoding the key enzymes involved in this process were determined by real time PCR during treatment with monoeoxyp-γ-tocotrienol (Table 3). Already after 15 min of such treatment,
the levels of mRNA species coding for COQ1 and COQ2, the enzymes that catalyze the first two steps in this process (one of which is considered to be rate-limiting) were elevated significantly. At the same time, the levels of mRNA for farnesyl-PP synthase and squalene synthase were not altered.

The Site of Inhibition of Cholesterol Synthesis—When HepG2 cells were incubated with [3H]mevalonate alone, radioactivity was recovered in cholesterol and in squalene, the first intermediate after the branch point (Fig. 5A). When the solanesol derivative containing two epoxide moieties was also present in the culture medium, labeling of cholesterol was greatly reduced, and in addition new radioactive peaks appeared in the HPLC chromatogram (Fig. 5B). Employing mass spectrometric analysis and authentic standards, these peaks were identified as 2,3-oxidosqualene (i.e. the substrate for oxidosqualene cyclase) and 2,3,22,23-dioxidosqualene and 24(S),25-epoxycholesterol (which is formed from 2,3,22,23-dioxidosqualene). Previous studies have demonstrated that these intermediates accumulate when oxidosqualene cyclase is inhibited (29, 30).

In order to analyze the site of inhibition in greater detail, RO 48-8071, an inhibitor of oxidosqualene cyclase, and ketoconazole, which inhibits lanosterol demethylase, the subsequent enzyme in cholesterol synthesis, were employed (31, 32) (Table 4). Tetraepoxysolanesol was used for comparison in these experiments, since this compound efficiently suppresses cholesterol synthesis. Incubation of the cells with either this epoxidated isoprenoid or RO 48-8071 eliminated labeling of cholesterol and resulted in the accumulation of labeled 2,3-oxidosqualene and 2,3,22,23-dioxidosqualene instead. In contrast, treatment of the cells with ketoconazole led to accumula-

**TABLE 4**

| Inhibitor   | Cholesterol  | 2,3-Oxidosqualene | 2,3,22,23-Dioxidosqualene | Lanosterol | Coenzyme Q<sub>10</sub> |
|-------------|--------------|--------------------|---------------------------|------------|-------------------------|
|             | cpm/pg protein | cpm/pg protein    | cpm/pg protein            | cpm/pg protein | cpm/pg protein |
| None        | 191 ± 12     | ND                 | 114 ± 25                  | ND         | 2.3 ± 0.2               |
| Tetraepoxysolanesol | ND           | 82 ± 8             | 91 ± 10                   | ND         | 3.0 ± 0.4               |
| RO 48-8071  | ND           | ND                 | 108 ± 22                  | ND         | 2.1 ± 0.2               |
| Ketoconazole| 25 ± 4<sup>a</sup> | ND                 | 58 ± 16                   | ND         | 2.4 ± 0.3               |

*<sup>a</sup> p < 0.001.

*FIGURE 5. Inhibition of cholesterol synthesis by solanesol epoxides.* HepG2 cells were cultured in the presence of 2 μM diepoxysolanesol for 24 h and thereafter labeled with [3H]mevalonate for 8 h. Subsequently, cellular lipids were extracted and analyzed by HPLC. A, in control cells, cholesterol is labeled (1), and the only intermediate observed is squalene (2). B, cells cultured in the presence of the diepoxysolanesol exhibit reduced labeling of cholesterol (1), and five additional radioactive peaks are seen, namely squalene (2), 24(S),25-epoxycholesterol (3), 2,3,22,23-dioxidosqualene (4), an unidentified intermediate (5), and 2,3-oxidosqualene (6). C, the distribution of cholesterol and its biosynthetic intermediates in HepG2 cells treated with 2 μM solanesol derivatives possessing 1–4 epoxide moieties. The individual columns illustrate the relative amounts in terms of percentages of the total amount of radioactivity recovered. D, the HPLC chromatogram of diepoxysolanesol reveals the presence of a complex mixture, reflecting the heterogeneous localization of the epoxides within the molecule.

Isoprenoid Epoxides Modulate CoQ and Cholesterol Synthesis

14650 JOURNAL OF BIOLOGICAL CHEMISTRY

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TABLE 5  
Biosynthesis of cholesterol from [3H]lanosterol by HepG2 cells in the presence of tetraepoxysolanesol, RO 48-8071, or ketoconazole

| Inhibitor         | Cholesterol incorporation a/µg protein |
|-------------------|--------------------------------------|
| None              | 10.5 ± 0.8                           |
| Tetraepoxysolanesol | 12.7 ± 1.5                           |
| RO 48-8071        | 10.1 ± 1.2                           |
| Ketoconazole      | 1.3 ± 0.3                            |

*p < 0.001.

The cells were cultured in the presence of 2 µM tetraepoxysolanesol or ketoconazole or 0.2 µM RO 48-8071 for 24 h, followed by incubation with [3H]lanosterol for an additional 8 h. Thereafter, the lipids were extracted and analyzed by HPLC employing on-line detection of radioactivity. The values presented are means ± S.E. of three independent experiments.

A more difficult problem involves the exact localization of the epoxide moieties within the side chain of solanesol and CoQ, since the chemical approach applied here yields a mixture of different distributions.

TABLE 6  
Effect of isoprenoid epoxides on the biosynthesis of CoQ and cholesterol synthesis by HepG2 cells

| Inhibitor         | Effect on CoQ synthesis | Effect on cholesterol synthesis |
|-------------------|-------------------------|---------------------------------|
| None              | -                       | -                               |
| Tetraepoxysolanesol | +                       | +                               |
| RO 48-8071        | +                       | +                               |
| Ketoconazole      | +                       | +                               |

*p < 0.001.

The effect of isoprenoid epoxides on the biosynthesis of CoQ and cholesterol synthesis by HepG2 cells was determined by measuring the incorporation of [3H]lanosterol into CoQ and cholesterol, respectively. The values presented are means ± S.E. of three independent experiments.

Discussion

The intermediates and end products of various biosynthetic processes, including cholesterol synthesis, often play a role in regulating these same processes, and our present goal was to search for such regulators of CoQ biosynthesis. Under the conditions employed here, UV irradiation of CoQ yielded a variety of compounds, and those containing epoxide moieties on the polyisoprene side chain were found to influence the mevalonate pathway in HepG2 cells. This observation led us to examine the effects of epoxidated forms of various polyisoprenoids in this same system.

More than 500 trans-trans-poly-cis-polyisoprenoids are known to occur naturally, ranging in size from a few isoprene units to several hundred, the latter forms being found in rubber (33). In contrast, naturally occurring all-trans-polysiprenoids are few in number, being present in smaller or larger quantities as farnesol, geranylgeraniol, solanesol, decaprenol, CoQ, vitamin K2, and tocotrienols. Moreover, since only certain epoxidated all-trans-polysiprenoids have been documented here to influence the biosynthesis of mevalonate pathway lipids, the number of potential regulatory substances is limited.

Interestingly, although epoxidated derivatives of short all-trans-polysiprenoids (i.e. farnesol and geranylgeraniol) exert little influence on lipid metabolism in HepG2 cells, tocotrienols with an epoxidated side chain exert marked effects on cholesterol and CoQ biosynthesis. These effects are highly dependent on the number of epoxide moieties present, necessitating isolation of the individual derivatives. A more difficult problem involves exact localization of the epoxide moieties within the side chain of solanesol and CoQ, since the chemical approach applied here yields a mixture of different distributions.

Approaches designed to enhance endogenous synthesis of CoQ are of considerable interest, since dietary supplementation is inefficient, with only a few percent of this lipid being taken up from the intestinal tract and transferred from the blood into the various organs. Unlike the situation with respect to cholesterol, no metabolic regulators of CoQ biosynthesis have yet been identified, although this synthesis is known to be up-regulated under certain conditions. In rodents, but not in humans, agonists of the nuclear receptor PPARα, including clofibrate, di(2-ethylhexyl)phthalate, and acetylsalicylic acid, both stimulate the biosynthesis of CoQ and elevate the level of this lipid in all organs, with the exception of the brain (34, 35). Among the hormones, thyroxine induces hepatic biosynthesis of CoQ and may be responsible for the elevated level of this lipid in the livers of rodents maintained at 4 °C for 10 days (36, 37). Furthermore, both vitamin A deficiency and inhibition of squa- lene synthase by squalestatin 1 lead to elevated levels of CoQ in both cultured cells and rats (38, 39).

Tocotrienols belong to the vitamin E family and are effective antioxidants in vitro. However, their antioxidant properties are not utilized in vivo, since the hepatic transfer protein is specific for α-tocopherol, and all other vitamin E compounds are excreted (40). On the other hand, a number of beneficial properties of exogenously administered tocotrienols have been described, including prevention of the adhesion of monocytes to endothelial cells, suppression of tumor cell growth, and inhibition of glutamate-induced neurotoxicity (41). It has also been proposed that γ- and δ-tocotrienols can attenuate cholesterol biosynthesis by stimulating the ubiquitination and consequent degradation of 3-hydroxy-3-methylglutaryl-CoA reductase, as well as by blocking the processing of proteins that bind to ste- rol-responsive elements in the promoters of genes (42).

Under our experimental conditions, the synthesis of cholesterol by HepG2 cells was not influenced by tocotrienols. In con- trast, tocotrienol epoxides exert profound effects on the syn-
Isoprenoid Epoxides Modulate CoQ and Cholesterol Synthesis

thesis of both CoQ and cholesterol. Tocotrienols are present in palm and rice oils, from which they can be purified in large quantities and stable form. Subsequent chemical epoxidation is highly efficient, and the resulting derivatives containing one or two epoxide moieties can be prepared with high purity. Even high concentrations of such epoxides are nontoxic toward cell cultures. Therefore, if polyisoprenoid epoxides influence CoQ synthesis in humans in a manner similar to that observed in cultures of HepG2 cells, these compounds may prove valuable for treating the CoQ deficiency associated with a number of diseases. In our on-going investigations on fibroblasts obtained from children carrying genetic defects in CoQ biosynthesis, tocotrienol epoxides have been found to induce the biosynthesis and increase the content of CoQ, indicating a possible strategy for treatment.

At present, treatment of hypercholesterolemia involves statins (i.e. inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase). Use of these drugs is based on the fact that squalene synthase, the initial enzyme in the terminal steps of cholesterol synthesis, demonstrates relatively low affinity for its substrate farnesyl-PP, whereas the branch-point enzymes for production of other mevalonate lipids exhibit higher affinities for this intermediate (43). As a consequence, the lower concentration of farnesyl-PP that results from statin treatment is insufficient to saturate squalene synthase, whereas the other committed enzymes remain saturated, and the levels of the other end products are not altered. However, a large number of studies involving both experimental animals and humans have revealed that both CoQ synthesis and protein isoprenylation are partially inhibited by treatment with statins, which may lead to undesirable side effects (17, 18).

Certain of the polyisoprenoid epoxides tested here are effective inhibitors of oxidosqualene cyclase, which catalyzes the formation of lanosterol. Accordingly, these modified compounds are of potential value for inhibiting the terminal reactions involved in cholesterol synthesis without inhibiting the synthesis of other mevalonate pathway lipids. Moreover, the tocotrienol derivatives containing two epoxide moieties both stimulate CoQ biosynthesis and inhibit cholesterol synthesis, which may be of special interest. In addition, inhibition of oxidosqualene cyclase leads to accumulation of 24(S),25-epoxycholesterol, which inhibits cholesterol synthesis by several mechanisms and thereby contributes to the overall inhibitory effect (44).

CoQ is considerably more sensitive to UV light than are other antioxidants and is degraded when skin preparations are exposed to such light (15). Thus, CoQ in free body surfaces exposed to intense UV light is also likely to undergo modification. A mixture of products, of which epoxidized forms represent a major portion, is formed in response to UV illumination. Earlier, Morimoto et al. (14) observed that irradiation of crystalline CoQ$_2$ with sunlight in the absence of solvent results in the formation of several compounds, including monooxepoxides. Thus, the modifications caused by UV light are much less drastic than the breakdown that can occur under other conditions. For example, lipid peroxidation in liposomes and beef heart submitochondrial particles is associated with fragmentation of the oxidized form of CoQ into a large number of more hydrophilic products (16).

In certain situations, such as in the human brain afflicted with Alzheimer’s disease, prion disease in the mouse brain, and in the liver and testis of diabetic rats, the level of CoQ is elevated. Moreover, dietary administration of CoQ$_{10}$ to rats and mice enhances their hepatic content of not only this compound itself (with its decaprenyl side chain) but also the endogenous CoQ$_{9}$ (45). The factor that stimulates the endogenous biosynthesis of CoQ under these circumstances may be an epoxide formed as a result of elevated oxidative stress or enhanced catabolism of CoQ. In fact, earlier studies have revealed the presence of small amounts of mono- and diepoxide derivatives of CoQ in beef heart mitochondria, *Rhodospirillum rubrum*, *Pseudomonas alkalanolyltica*, and the heart muscle of whales (46–48). We are presently examining possible correlations between the presence of epoxidated polyisoprenoids and enhanced CoQ biosynthesis in various systems.

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