Effects of Atovaquone and Diospyrin-Based Drugs on Ubiquinone Biosynthesis in Pneumocystis carinii Organisms

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Received 8 March 1999/Returned for modification 21 September 1999/Accepted 8 October 1999

The naphthoquinone atovaquone is effective against Plasmodium and Pneumocystis carinii carinii. In Plasmodium, the primary mechanism of drug action is an irreversible binding to the mitochondrial cytochrome bc1 complex as an analog of ubiquinone. Blockage of the electron transport chain ultimately inhibits de novo pyrimidine biosynthesis since dihydroorotate dehydrogenase, a key enzyme in pyrimidine biosynthesis, is unable to transfer electrons to ubiquinone. In the present study, the effect of atovaquone was examined on Pneumocystis carinii carinii coenzyme Q biosynthesis (rather than electron transport and respiration) by measuring its effect on the incorporation of radiolabeled p-hydroxybenzoate into ubiquinone in vitro. A triphasic dose-response was observed, with inhibition at 10 nM and then stimulation up to 0.2 μM, followed by inhibition at 1 μM. Since other naphthoquinone drugs may also act as analogs of ubiquinone, diospyrin and two of its derivatives were also tested for their effects on ubiquinone biosynthesis in P. carinii carinii. In contrast to atovaquone, these drugs did not inhibit the incorporation of p-hydroxybenzoate into P. carinii carinii ubiquinone.

Ubiquinone (coenzyme Q [CoQ]) (Fig. 1A) plays a pivotal role in cellular respiration by participating in inner mitochondrial membrane electron transport by accepting electrons from a number of dehydrogenase enzymes and passing them to the cytochromes and eventually to molecular oxygen (6, 8, 30). Biosynthesis of ubiquinone (1, 22, 26, 29, 33) and the reactions that occur in different cellular compartments are probably best understood from studies with subcellular fractions of rat liver cells (1, 22, 29). Ubiquinone is composed of a benzoquinone ring and a lipophilic polyprenyl chain. In the rat liver, the precursor of the benzoquinone moiety, p-hydroxybenzoic acid (PHBA), is formed in the cytosol from the aromatic amino acids tyrosine or phenylalanine, whereas PHBA is formed directly from chorismic acid in organisms that have the shikimic acid pathway (i.e., plants, bacteria, and some fungi). The polyprenyl chain of CoQ is formed by a branch pathway in isoprenoid biosynthesis by the polymerization of five-carbon isopentenyl units; the number of the isopentenyl units in the chain distinguishes the CoQ homologs. The major CoQ homolog has been used as a taxonomic criterion for the verification of the phylogenetic relationships between organisms (32). There appears to be two independent sites where ubiquinone is synthesized in eukaryotic cells: one is the endoplasmic reticulum (ER)-Golgi system and the other is the mitochondrion (13, 33). After the transfer of a polyprenyl P-P chain to PHBA, followed by several additional reactions (1, 22, 26, 29, 33), the completed CoQ homolog is produced.

Purified Pneumocystis carinii carinii (P. carinii) isolated from methylprednisolone-immunosuppressed rats was shown to contain CoQ10 as the major CoQ homolog (smaller amounts of CoQ9 were also detected) (10). Since CoQ10 was not detected in the lungs of healthy, untreated or in the lungs of immunosuppressed, un inoculated rat controls, this suggested that the pathogen was at least capable of synthesizing CoQ10. Recently, the incorporation in vitro of chorismate, PHBA, tyrosine, and mevalonate into P. carinii CoQ was demonstrated (10, 24, 28); thus, this organism can synthesize de novo both moieties of ubiquinone. The P. carinii pentafunctional gene for enzymes in the shikimic acid pathway has been cloned and characterized (2), suggesting that the pathway is functional in this organism. This gene is localized in the nucleus; hence, it is likely that ubiquinone biosynthesis in this organism occurs in the ER-Golgi system, although synthesis in the mitochondria cannot be ruled out.

Several hydroxynaphthoquinone drugs that are effective against protozoan infections (e.g., malaria, trypanosomiasis, and leishmaniasis) also have activity against P. carinii. Atovaquone (Fig. 1), first used as an antimalarial agent, was also found to have therapeutic activity against P. carinii pneumonia (PCP). This has been demonstrated both in animal models (19) and in humans (7, 14, 20). The mechanism of action of atovaquone against Plasmodium is believed to result from the irreversible binding of the drug to a 11.5-kDa protein of the mitochondrial cytochrome bc1 complex, thus inhibiting electron transport (12, 14–16). Since dihydroorotate dehydrogenase (DHOD), a key step in de novo pyrimidine synthesis, is coupled to the electron transport chain at complex III and because the parasite cannot salvage host pyrimidines, the mechanism of cidal drug action is thought to be the blockage of pyrimidine biosynthesis as a consequence of electron transport inhibition.

It was previously reported that the 50% inhibitory concentration (IC50) for P. carinii O2 consumption was 5 × 10−6 M atovaquone (14, 15). Thus, the respiratory chain was also implicated as the site of action in P. carinii. It was hypothesized, however, that unlike Plasmodium, P. carinii could salvage host pyrimidines, and the depletion of ATP (resulting from inhibition of respiration) was proposed as a mode by which P. carinii is killed by the drug (15). Moreover, unlike Plasmodium DHOD activity, which is inhibited by 1 nM atovaquone (12), the activity of this enzyme in P. carinii lysates was not inhibited by concentrations of ≤10 μM (21). Although atovaquone and other hydroxynaphthoquinone drugs are recognized as ubiquinone analogs, details on the mechanisms of their antimicrobial activities in different organisms remain unclear.

To test the hypothesis that oxidative phosphorylation is...
highly active in the respiratory chain of *P. carinii* and that the consequence of the drug’s efficacy against PCP is the disruption of ATP synthesis, direct measurements of cellular ATP were performed. The effect of atovaquone on the ATP content of *P. carinii* organisms is described in a separate report (M. T. Cushion, et al., submitted for publication). In the present study, the effect of atovaquone on CoQ biosynthesis in *P. carinii* was examined by the incorporation in vitro of radiolabeled PHBA into CoQ. The results were compared with those obtained with another group of naphthoquinoid drugs which appear to be promising as antiparasitic agents (Fig. 1). Diospyrin, a natural product of Diospyros montana stem bark, and two of its derivatives (17, 18) exhibit activity in vitro against *P. carinii*, *Leishmania*, and *Trypanosoma* at micromolar concentrations (18, 34). In an attempt to better understand the mechanism of antiparasitic activities of different quinoid drugs, these compounds were also examined for their effects on ubiquinone biosynthesis in *P. carinii*.

**MATERIALS AND METHODS**

Organisms. *P. carinii* was isolated from infected rat lungs by using the corticosteroid-immunosuppressed rat model of Boylan and Current (4). Viral anti-body-negative female Lewis rats (Harlan Sprague-Dawley, Indianapolis, Ind.) were immunosuppressed with methylprednisolone acetate (Depe-Medrol; Upjohn Co., Kalamazoo, Mich.) and were twice inoculated intratraehicularly with cryopreserved organisms containing 10⁶ to 10⁷ mixed-life-cycle stages by previ-bodies-negative female Lewis rats (Harlan Sprague-Dawley, Indianapolis, Ind.) costeroid-immunosuppressed rat model of Boylan and Current (4). Viral anti-Diospyrin (R

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FIG. 1. Structures of compounds relevant to the present study. (A) Ubiqui-nine (CoQ); n, number of isoprenyl units. (B) Atovaquone (556C80). (C) Diospyrin (R = H) and diospyrin dimethylether (R = CH₃). (D) Diospyrin dimethylether hydroquinone (R = CH₃).

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cous concentrations of each drug were added to radiolabeled PHBA before the organisms, suspended in the RPMI 1640 medium, were introduced into the re-action mixture. The ethanol concentration in the final incubation mixture was <0.1%, and the DMSO concentration was 0.1%.

**Extraction of lipids and determination of *P. carinii* CoQ radioactivity.** The lipid extraction, purification, and fractionation methods used for studies on the incorporation in vitro of radiolabeled precursors into *P. carinii* ubiquinones were as described previously (28). Briefly, total lipids were extracted by a neutral solvent system as described by Bligh and Dyer (3) and were purified by biphasic partitioning as described by Folch et al. (11). The neutral lipid fraction was obtained by adsorption column chromatography (Unisil; Clarkson Co., Williams-port, Pa.), by elution with chloroform (CHCl₃) and was then resolved by 1-di-mensional thin-layer chromatography (TLC) on 0.25-mm Silica Gel H glass-plates (Analytech, Inc., Newark, Del.) prewashed with methanol. The TLC plates were developed with the solvent system petroleum ether-dichloroethane-acetic acid (80:20:1; vol/vol/vol) (9, 10). After visualization with I₂ vapor, the ubiquinone band was scraped off the TLC plate and its radioactivity was determined by liquid scintillation spectrometry.

Incorporation of PHBA into the *P. carinii* total ubiquinone TLC fraction was expressed as picomoles of PHBA per milligram of protein from the original organism preparations. The effects of the drugs on CoQ biosynthesis were com-pared to those of vehicle controls.

**RESULTS**

Effects of atovaquone on the incorporation in vitro of PHBA into *P. carinii* ubiquinones. Incorporation of PHBA into *P. carinii* CoQ exhibited a triphasic response to increasing atovaquone concentrations (Table 1; Fig. 2). A dramatic inhibi-tion of CoQ biosynthesis was detected at 10 nM, an effect that was reproducibly observed. At this concentration, incorporation was less than 50% of that for the untreated controls. At between 0.1 and 0.2 μM, incorporation occurred at levels comparable to or higher than those for the controls. The high-er IC₅₀ in the triphasic dose-response curve was observed at 1.0 μM.

Effects of diospyrin and its derivatives on the incorporation of PHBA into *P. carinii* ubiquinones. Diospyrin, diospyrin di-methylether, and diospyrin dimethylether hydroquinone did not inhibit the incorporation in vitro of PHBA into *P. carinii* ubiquinones when incubation was done for 48 h at concentra-tions up to 100 μM (Table 1; Fig. 3); dose-dependent reduc-tions in CoQ biosynthesis were not observed.

**DISCUSSION**

Effects of atovaquone on *P. carinii* metabolism and respira-tion. Atovaquone is effective in clearing organisms from patients with PCP with low to moderate numbers of organisms (7, 12, 20). It was previously reported that the drug inhibited *P. carinii* respiration (measured polarographically) at an IC₅₀ of 50 nM (14, 15). Since it is known that atovaquone’s activity in *Plasmodium* is the consequence of binding to the mitochondri-al cytochrome bc₁ complex (13–15) and that it apparently also binds to the *P. carinii* bc₁ complex (14), it is not surprising that inhibition of *P. carinii* respiration was observed.

The present study addresses the effect of atovaquone other than electron transport and respiration. Atovaquone had a triphasic dose-effect on PHBA incorporation into *P. carinii* ubiquinone. Inhibition was observed at 10 nM atovaquone (the lowest concentration tested), which was lower than the con-centration reported to be required for detectable inhibition of respiration (14), reduction of cellular ATP (Cushion et al., submitted), inhibition of DHOD activity, or inhibition of organ-ism proliferation in primary cultures (21).

In a previous study, CoQ₇ and CoQ₈ were not detected by high-pressure liquid chromatography (HPLC) and/or gas-liquid chromatography–mass spectrometry (10) methods, indi-cating that these homologs do not accumulate to readily detectable levels in the organism. By using more sensitive met abolic radiolabeling techniques, it was recently found that the
TABLE 1. Effects of naphthoquinone drugs on incorporation in vitro of radiolabeled p-hydroxybenzoate into *P. carinii* ubiquinone*<sup>c</sup>*

| Drug and concn (μM) | Radioactivity (dpm)* | Incorporation of PHBA into CoQ (pmol/mg of protein)* |
|---------------------|----------------------|-----------------------------------------------------|
| Atovaquone*<sup>d</sup> |                      |                                                     |
| 0                   | 5,221–5,684          | 151.8 ± 2.4                                         |
| 0.01                | 2,037–2,516          | 59.3 ± 2.0                                          |
| 0.02                | 3,263–3,453          | 92.8 ± 1.0                                          |
| 0.1                 | 3,815–5,668          | 170.5 ± 4.3                                         |
| 0.2                 | 5,205–6,105          | 155.3 ± 5.4                                         |
| 1                   | 2,458–2,905          | 74.3 ± 2.1                                          |
| Diospyrin*<sup>e</sup> |                      |                                                     |
| 0                   | 525–1,037            | 66.0 ± 4.3                                          |
| 0.01                | 468–1,105            | 58.1 ± 6.5                                          |
| 0.05                | 495–832              | 56.2 ± 3.3                                          |
| 0.1                 | 389–974              | 56.7 ± 5.1                                          |
| 0.5                 | 411–1,042            | 56.5 ± 8.0                                          |
| 1                   | 468–1,168            | 65.5 ± 6.3                                          |
| 5                   | 474–1,200            | 63.2 ± 7.8                                          |
| 10                  | 498–1,168            | 66.5 ± 6.2                                          |
| 25                  | 403–1,037            | 59.9 ± 6.0                                          |
| 50                  | 394–921              | 58.6 ± 4.6                                          |
| 100                 | 442–1,105            | 60.6 ± 6.5                                          |
| Diospyrin dimethylether<sup>e</sup> |          |                                                     |
| 0                   | 252–397              | 48.2 ± 3.7                                          |
| 0.01                | 205–242              | 45.9 ± 1.9                                          |
| 0.05                | 237–435              | 53.9 ± 5.6                                          |
| 0.1                 | 232–446              | 46.2 ± 2.5                                          |
| 0.5                 | 137–382              | 46.7 ± 7.7                                          |
| 1                   | 177–347              | 43.9 ± 5.2                                          |
| 5                   | 226–279              | 51.5 ± 2.6                                          |
| 10                  | 247–421              | 49.3 ± 4.8                                          |
| 25                  | 253–381              | 46.6 ± 4.9                                          |
| 50                  | 242–392              | 45.8 ± 3.4                                          |
| 100                 | 221–432              | 46.4 ± 4.6                                          |
| Diospyrin dimethylether hydroquinone<sup>e</sup> | | |
| 0                   | 274–689              | 58.2 ± 10.9                                         |
| 0.01                | 523–679              | 79.6 ± 5.0                                          |
| 0.05                | 295–453              | 63.9 ± 9.1                                          |
| 0.1                 | 342–568              | 56.2 ± 6.3                                          |
| 0.5                 | 418–621              | 63.0 ± 7.4                                          |
| 1                   | 379–584              | 53.2 ± 6.4                                          |
| 5                   | 442–668              | 77.2 ± 7.5                                          |
| 10                  | 368–768              | 62.4 ± 10.6                                         |
| 25                  | 354–820              | 61.1 ± 12.0                                         |
| 50                  | 361–558              | 56.4 ± 7.2                                          |
| 100                 | 279–515              | 48.5 ± 5.4                                          |

*a* Purified organisms were incubated for 2 days in 10 ml of serum-free RPMI 1640 medium containing [U-14C]p-hydroxybenzoate and various concentrations of a drug. Values are means ± standard errors of the means.

*b* Values are ranges of radioactivity in the ubiquinone band isolated by TLC.

*c* Protein content of organisms prior to incubation.

*d* Organisms were incubated with 10 μCi of the substrate (*n* = 4).

*e* Organisms were incubated with 5 μCi of the substrate (*n* = 6 except in the assays with 0.01 and 5 μM, in which *n* = 3).

HPLC fractions eluting with authentic CoQ<sub>7</sub>, CoQ<sub>8</sub>, CoQ<sub>9</sub>, and CoQ<sub>10</sub> were all radioactive (D. Sul et al., unpublished data). The high specific activity of the shorter homologs and the conversion of radiolabeled CoQ<sub>8</sub> to CoQ<sub>9</sub> and CoQ<sub>10</sub> suggest that the longer homologs can be formed by elongation of the polyprenyl chains of completed CoQ molecules. The biosynthesis of CoQ homologs by elongation of CoQ polyprenyl chains would represent a novel mechanism for CoQ<sub>10</sub> biosynthesis.

Information on the regulatory mechanisms that control steps in CoQ biosynthesis in any cell type is severely lacking in the literature. On the basis of the available data on *P. carinii* and what is currently known about ubiquinone biosynthesis in general, we propose the following hypotheses or scheme as a working model to explain the observations on atovaquone’s effect on *P. carinii* (Fig. 4). (i) At the low (10 nM) concentration, as an analog of CoQ, atovaquone may inhibit de novo CoQ biosynthesis by activating putative product feedback mechanisms that reduce the incorporation of PHBA into CoQ. In untreated cells, the enzyme would be regulated by the ac...
atovaquone, the drug acts as an analog of CoQ10 and inhibits the PHBA-polyprenyltransferase activity. At 10 nM units may occur at the outer surface of the ER, and then heptaprenyl P-P is the cytosol. Elongation of the polyprenyl precursor by the addition of isopentenyl effects of atovaquone (Av). The precursors PHBA and geranyl P-P are formed in completed polyprenyl chains could be formed prior to condensation which accumulates in the organism (10). Alternatively, the organelle from the cytosol) condenses with heptaprenyl ER-Golgi, where PHBA (which has also been transported into the membrane, forming quinol and quinone pools in the membrane (6, 30). Thus, atovaquone is effective as a ubiquinone analog at triggering this feedback control of PHBA-polyprenyltransferase activity. (ii) At concentrations between 20 nM and 0.2 μM, atovaquone competes for sites on a carrier and/or binds to some (but not all) cytochrome bc1 complexes in the mitochondrial inner membrane, resulting in a reduction in the level of electron transport. At these concentrations, atovaquone may displace and prevent the binding of ubiquinone from some cytochrome bc1 complexes; i.e., the drug binds irreversibly to some of the bc1 complexes present in the membrane. This would result in detectable inhibition of respiration (decreased respiration in P. carinii carinii was detected with atovaquone at 50 nM [14, 15]). The inhibition of respiration may then trigger upregulation of the biosyntheses of components of CoQ intracellular transport (e.g., carrier) and/or the electron transport chain (e.g., ubiquinone) in response to the need to increase the cell’s respiratory capacity. The upregulation of these biosynthetic activities might override the negative, end product feedback control(s) which atovaquone, at lower concentrations, could activate as a ubiquinone analog. Thus, with atovaquone at between 20 nM and 0.2 μM, CoQ biosynthesis (incorporation of PHBA into P. carinii CoQ) is increased to normal or higher levels. However, at these drug concentrations, ample electron transport could be maintained by CoQ molecules still in place within the mitochondrial inner membrane; thus, O2 consumption is only slightly affected, and the inhibition of oxidative phosphorylation, as measured by ATP levels in the cell, is not detectable (ATP pools may also be maintained by synthesis in the glycolytic pathway). (iii) At the higher drug concentrations (>1 μM), we hypothesize that sufficient amounts of atovaquone become irreversibly bound to most cytochrome bc1 complexes in the mitochondrial membrane. This would result in detectable inhibition of respiration (decreased respiration in P. carinii carinii and 0.2 μM, atovaquone competes for sites on a carrier and/or electron transport (e.g., ubiquinone), which can override the negative, end product feedback control(s). With atovaquone at concentrations of >1 μM, respiration is sufficiently inhibited and the reduction in ATP production by oxidative phosphorylation becomes measureable. The lack of ATP causes a decline in overall cellular metabolism, resulting in a decrease in the rate of PHBA incorporation into CoQ. Ca, carrier.

FIG. 4. Proposed scheme for ubiquinone biosynthesis in P. carinii and the effects of atovaquone (Av). The precursors PHBA and geranyl P-P are formed in the cytosol. Elongation of the polyprenyl precursor by the addition of isopentenyl units may occur at the outer surface of the ER, and then heptaprenyl P-P is translocated to the ER and then to the Golgi apparatus lumen. At 10 nM atovaquone, the drug acts as an analog of CoQ10 and inhibits the PHBA-polyprenyltransferase activity (→), reducing the incorporation of PHBA into CoQ. In this model, it is proposed that decreased respiration stimulates the upregulation of biosyntheses of components involved in the intracellular translocation of CoQ (e.g., carrier) and/or electron transport (e.g., ubiquinone), which can override the negative, end product feedback control(s). With atovaquone at concentrations of >1 μM, respiration is sufficiently inhibited and the reduction in ATP production by oxidative phosphorylation becomes measurable. The lack of ATP causes a decline in overall cellular metabolism, resulting in a decrease in the rate of PHBA incorporation into CoQ. Ca, carrier.

cumulation of free CoQ (CoQ10). This probably occurs at a cellular site closely associated with the mitochondrion or the ER-Golgi, where PHBA (which has also been transported into the organelle from the cytosol) condenses with heptaprenyl P-P or octaprenyl P-P. Following several reactions, including three S-adenosylmethionine (SAM)-dependent methyltransfer steps, the intermediate is converted to the completed CoQ7 molecule (a homolog radiolabeled with CoQ precursors in P. carinii). The completed CoQ product is translocated to the cytosolic side of the Golgi (or ER) membrane, where elongation of the polyprenyl chain occurs by the sequential addition of isopentenyl units, producing the major homolog CoQ10 which accumulates in the organism (10). Alternatively, completed polyprenyl chains could be formed prior to condensation with PHBA. In this scheme, it is proposed that feedback control involving inhibition by accumulation of CoQ10 in a free pool decreases the PHBA-hexaprenyltransferase activity in the Golgi-ER system. Translocation of CoQ10 to the inner mitochondrial membrane may require binding to a carrier protein which can target it to the mitochondrion. In the mitochondrion, CoQ participates in electron transport and interacts with the membrane, forming quinol and quinone pools in the membrane (6, 30). Thus, atovaquone is effective as a ubiquinone analog at triggering this feedback control of PHBA-polyprenyltransferase activity. (ii) At concentrations between 20 nM and 0.2 μM, atovaquone competes for sites on a carrier and/or binds to some (but not all) cytochrome bc1 complexes in the mitochondrial inner membrane, resulting in a reduction in the level of electron transport. At these concentrations, atovaquone may displace and prevent the binding of ubiquinone from some cytochrome bc1 complexes; i.e., the drug binds irreversibly to some of the bc1 complexes present in the membrane. This would result in detectable inhibition of respiration (decreased respiration in P. carinii carinii was detected with atovaquone at 50 nM [14, 15]). The inhibition of respiration may then trigger upregulation of the biosyntheses of components of CoQ intracellular transport (e.g., carrier) and/or the electron transport chain (e.g., ubiquinone) in response to the need to increase the cell’s respiratory capacity. The upregulation of these biosynthetic activities might override the negative, end product feedback control(s) which atovaquone, at lower concentrations, could activate as a ubiquinone analog. Thus, with atovaquone at between 20 nM and 0.2 μM, CoQ biosynthesis (incorporation of PHBA into P. carinii CoQ) is increased to normal or higher levels. However, at these drug concentrations, ample electron transport could be maintained by CoQ molecules still in place within the mitochondrial inner membrane; thus, O2 consumption is only slightly affected, and the inhibition of oxidative phosphorylation, as measured by ATP levels in the cell, is not detectable (ATP pools may also be maintained by synthesis in the glycolytic pathway). (iii) At the higher drug concentrations (>1 μM), we hypothesize that sufficient amounts of atovaquone become irreversibly bound to most cytochrome bc1 complexes in the mitochondrial membrane. This would result in detectable inhibition of respiration (decreased respiration in P. carinii carinii and 0.2 μM, atovaquone competes for sites on a carrier and/or electron transport (e.g., ubiquinone), which can override the negative, end product feedback control(s). With atovaquone at concentrations of >1 μM, respiration is sufficiently inhibited and the reduction in ATP production by oxidative phosphorylation becomes measurable. The lack of ATP causes a decline in overall cellular metabolism, resulting in a decrease in the rate of PHBA incorporation into CoQ. Ca, carrier.

cumulation of free CoQ (CoQ10). This probably occurs at a cellular site closely associated with the mitochondrion or the ER-Golgi, where PHBA (which has also been transported into the organelle from the cytosol) condenses with heptaprenyl P-P or octaprenyl P-P. Following several reactions, including three S-adenosylmethionine (SAM)-dependent methyltransfer steps, the intermediate is converted to the completed CoQ7 molecule (a homolog radiolabeled with CoQ precursors in P. carinii). The completed CoQ product is translocated to the cytosolic side of the Golgi (or ER) membrane, where elongation of the polyprenyl chain occurs by the sequential addition of isopentenyl units, producing the major homolog CoQ10 which accumulates in the organism (10). Alternatively, completed polyprenyl chains could be formed prior to condensa-
there may be several possible mechanisms of action of the drug on this pathogen, and these mechanisms may also occur with other organisms, such as Toxoplasma and Plasmodium. Atova-
quone-resistant strains have been identified among these or-
ganisms. Mutations in the cytochrome b gene appear to explain
the development of some P. carinii-resistant strains (31). Since
atovaquone was found to have a profound effect on other
processes besides electron transport, it is possible that atova-
quone resistance may also involve changes in components that
function in the biosynthesis or intracellular translocation of
ubiquinone.

Effects of diospyrin, diospyrin dimethylether, and diospyrin
dimethyldioxyhydroquinone on P. carinii ubiquinone biosyn-
thesis. It has been suggested that the mechanisms of action
of quinoid drugs with antiparasite activity, as well as quinoid
metabolites of some other drugs (e.g., primaquine), are by
their action as analogs of ubiquinone (8, 15). Since diospyrin
its metabolites of some other drugs (e.g., primaquine), are by
the yeast genera Candida and Pichia, which are by
quinoceptor activity. Additional studies on the effects of the
diospyrin-based quinoid drugs. Additional studies on the effects of the
biosynthetic rates, this strongly suggests that the mechanism of
action as analogs of ubiquinone (8, 15). Since diospyrin
quione resistance may also involve changes in components that
processes besides electron transport, it is possible that atova-
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