NEW QUINOLINEQUINONE INHIBITORS OF MITOCHONDRIAL REDUCTASE SYSTEMS AND REVERSAL BY COENZYME Q

by

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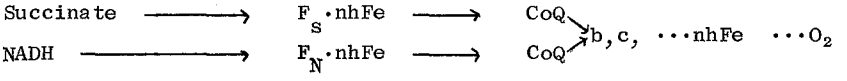
Summary

3-ω-Cyclohexyloctyl-2-hydroxy-1,4-naphthoquinone and 7-ω-cyclohexyloctyl-6-hydroxy-5,8-quinolinequinone inhibit the succinate-cytochrome c reductase and the succinate-coenzyme Q reductase in beef heart submitochondrial systems. These inhibitions could be reversed by coenzyme Q_6. These analogs of coenzyme Q also inhibit the succinate-coenzyme Q reductase of intact mitochondria from the human heart.

It is now known that there are two sites for the function of coenzyme Q in mitochondrial electron tramsport as exemplified:



This knowledge is essentially based upon the organic structural specificity of CoQ for activity in the succin-oxidase and NADH-oxidase systems of beef heart[1,2] and yeast mitochondria[2,3]. It is interesting that where increasing isoprenoid side-chain lengths of members of the CoQ group enhance activity of NADH-oxidase in beef heart mitochondria[1], shorter side-chain lengths appear to increase activity of NADH-oxidase in yeast mitochondria[2]. There appears to be little specificity for the side-chain length in the succinoxidase system. A recent study by Ernster et al.[3] indicates that CoQ may act as a regulator of the interaction of NADH and succinate dehydrogenase with the cytochrome system.

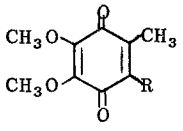
CoQ has been found, recently, in the malarial parasite (i.e., Plasmodium knowlesi, P. cynomolgi, P. lophurae, P. berghei, and P. falciparum). CoQ_8, I, was found to be the dominant form of CoQ in the parasite as determined both by

*Coenzyme Q. CXXXIV.

a differential analysis of normal and infected blood[4,5] and by a ^{14}C-labeling technique using in vitro cultures of erythrocytes infected with P. knowlesi[6] and P. falciparum[7].

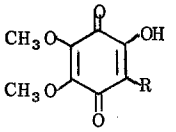
Since CoQ is intrinsically involved in oxidative metabolism and has been found to occupy two sites in the electron transfer chain, the design of specific CoQ inhibitors of either the function and/or the biosynthesis of CoQ in Plasmodium is a new approach to the chemotherapy of malaria.

Naphthoquinones[8] have shown significant antimalarial activity. Two such compounds, III and IV, are known to be active against P. vivax in the human. Recently, it was found that certain antimalarial naphthoquinones and some new hydroxy analogs, II, of CoQ do inhibit the mammalian CoQ enzyme systems[9].

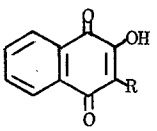


I

R=$(CH_2-CH=\overset{CH_3}{C}-CH_2)_8H$

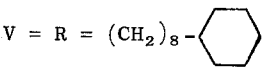


II



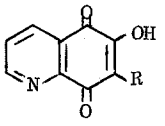
III = R = $(CH_2)_3$-⬡

IV = R = $(CH_2)_8-\overset{H}{\underset{OH}{C}}-(C_5H_{11})_2$



V = R = $(CH_2)_8$-⬡

We have now synthesized and studied the inhibition of the succinate-cytochrome c and succinate-coenzyme Q reductases by the following new exemplary 5, 8-quinolinequinone analogs, VI and VII, of the naphthoquinone antimalarials. These quinolinequinones are more effective inhibitors of the coenzyme Q reduc-



VI = R = $-(CH_2)_{14}CH_3$

VII = R = $-(CH_2)_8$-⬡

tase systems than the corresponding 1,4-naphthoquinones. The inhibitions can be completely reversed by exogenous addition of CoQ_6 to the beef heart submitochondrial systems. For example[10], the inhibition of succinate-cytochrome c reductase by 0.75 nm of 2-cyclohexyloctyl-3-hydroxy-1,4-naphthoquinone was completely reversed by the addition of 3.0 nm of coenzyme Q_6.

The availability of effective inhibitors of CoQ and knowledge in the inhibition of CoQ and reversal may lead to useful results in areas of chemotherapy other than malaria.

MATERIALS AND METHODS

Protein was determined by the method of Lowry et al.[11] using the Folin phenol reagent. Cytochrome c from horse heart and NADH were obtained from the Sigma Chemical Co., St. Louis, Missouri.

Preparation of Beef Heart Mitochondria. - Heavy beef heart mitochondria (HBHM) were prepared by a modified method of Löw and Vallin[12]. The final mitochondrial pellet was suspended in a medium which was 0.25 M in sucrose and 0.01 M in tris-HCl, pH 7.5. The suspension was kept cold in an ice bath, homogenized by means of a power driven Potter-Elvehjem homogenizer, and centrifuged at 18,800 rpm (25,000 g) in a Spinco ultracentrifuge (60-Ti rotor) for ten minutes. The heavy layer was then resuspended in 0.25 M sucrose, homogenized, and adjusted to 30 mg of protein per ml of buffer.

Preparation of Beef Heart Submitochondrial Particles[13]. - The ice cold HBHM suspension (adjusted to 30 mg protein) was subjected to ultrasonic oscillation at maximum output for four minutes (with intermittent cooling every other minute). The suspension was centrifuged at 13,000 rpm (12,000 g) in the Spinco ultracentrifuge (60-Ti rotor) for 10 minutes. The supernatant (separated from pellet) was centrifuged at 38,000 rpm (101,000 g) for 40 minutes. The pellet was washed by homogenization with 0.25 M sucrose and recentrifugation. It was then resuspended in the same buffer to a protein concentration of 30 mg/ml.

Preparation of Intact Human Heart Mitochondria. - The conditions of homogenization and isolation of mitochondria were based on the methods of Smith[14] and of Ernster and Nordenbrand[15]. Autopsy tissue from a normal human heart atrium which showed no deficiency in CoQ_{10}[16] was added to 5 ml of a solution which was 0.01 M tris-HCl buffer, pH 7.8, and 0.25 M in sucrose before mechanical homogenization. The homogenate was filtered through triple-layered 44/36 mesh gauze prior to centrifugation in 30 ml of the sucrose-tris solution for 20 minutes at 4,000 rpm in a Beckman ultracentrifuge (60-Ti rotor). The supernatant was decanted, filtered through gauze vide supra and recentrifuged at 19,000 rpm (27,000 g) for 20 minutes. The mitochondrial pellet was resuspended in 30 ml of the sucrose-tris solution which contained 1% of bovine serum albumin. The mixture was recentrifuged at 27,000 g for 20 minutes and washed again with 30 ml of the sucrose-tris solution. The heavy layer was resuspended in 0.25 M sucrose to a protein concentration of 3.36 mg/ml.

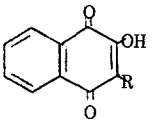
Succinate-Cytochrome c Reductase. - The procedure of Tisdale[17] was used to follow the reduction of cytochrome c at 550 mμ. The sample cuvette (1 ml) contained 0.10 ml potassium phosphate buffer (10 μM); 0.01 ml NaN_3 (1 μM); 0.02 ml EDTA (0.2 μM); 0.1 ml ferricytochrome c (1 mg); 0.10 ml potassium succinate (10 μM, pH 7.0) and water to a volume of 0.9 ml. After equilibration at 37°, the reaction was initiated by adding 0.02 ml of submitochondrial particles, diluted to a concentration of 0.1-1.0 mg/ml in a solution of 0.88 M sucrose and 0.005 M in succinate.

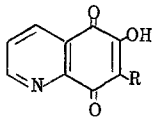
Succinate-Coenzyme Q Reductase. - The rapid rate of reduction of 2,6-dichloroindophenol by H_2CoQ was followed spectrophotometrically at 600 mμ by the

method of Ziegler and Rieske[18]. The sample cuvette (1 ml) contained 0.1 ml phosphate buffer (10. M, pH 7); 0.015 ml indophenol solution (0.1%); 0.02 ml potassium succinate (1.0 M, pH 7); 0.01 ml EDTA (1.0 mM); 0.001 ml of Triton

TABLE I

INHIBITION OF SUCCINATE CYTOCHROME c REDUCTASE
BY QUINONES IN BEEF HEART SUBMITOCHONDRIAL PARTICLES





Compound	Conc. (μmoles/mg protein)	Specific Activity (μmoles of cyt. c red./min./mg protein)
	none	0.25
	7.0×10^{-4}	0.20
V	9.0×10^{-4}	0.10
R = $-(CH_2)_8C_6H_{11}$	1.4×10^{-3}	0.04
	1.8×10^{-3}	0.02
	none	0.31
VII	1.4×10^{-3}	0.18
R' = $-(CH_2)_8C_6H_{11}$	1.8×10^{-3}	0.09

INHIBITION OF SUCCINATE-COENZYME Q REDUCTASE
BY QUINONES IN BEEF HEART SUBMITOCHONDRIAL PARTICLES

	Conc. (μmoles/mg protein)	Specific Activity (μmoles of indophenol red./min./mg protein)
	none	0.30
	2.88×10^{-2}	0.23
V	4.32×10^{-2}	0.16
R = $-(CH_2)_8C_6H_{11}$	5.76×10^{-2}	0.11
	none	0.25
VII	1.44×10^{-2}	0.18
R' = $-(CH_2)_8C_6H_{11}$	2.88×10^{-2}	0.13

INHIBITION OF SUCCINATE-COENZYME Q REDUCTASE
BY QUINONES IN INTACT HUMAN HEART MITOCHONDRIA

	Conc. (μmoles/mg protein)	Specific Activity (mμmoles of indophenol red./min./mg protein)
	none	110
	4.2×10^{-2}	65
V	8.3×10^{-2}	61
R = $-(CH_2)_8C_6H_{11}$	1.3×10^{-1}	54
	none	110
VI	1.5×10^{-2}	57
R' = $-(CH_2)_{14}CH_3$	4.5×10^{-2}	32
	none	110
VII	1.8×10^{-2}	61
R' = $-(CH_2)_8C_6H_{11}$		

X-100 (10%); 0.002 ml of CoQ_6 solution (5 mg/ml in ethanol) in the beef heart experiments and 3.0 mμmol of CoQ_3 in the human heart experiments, and water to bring the total volume to 1.0 ml.

After temperature equilibration at 37°, the reaction was started in one case by the addition of 0.1-1.0 mg of beef heart submitochondrial protein. In the second case, the reaction was started by the addition of 0.67 mg of intact human heart mitochondria.

RESULTS AND DISCUSSION

The inhibition of succinate-cytochrome c reductase by both 3-ω-cyclohexyloctyl-6-hydroxy-5,8-quinolinequinone (VII) and 3-ω-cyclohexyloctyl-2-hydroxy-1,4-naphthoquinone (V) was observed in a heavy beef-heart submitochondrial system (Table I). The naphthoquinone was somewhat more inhibitory than the quinolinequinone.

The inhibition of mitochondrial succinate-coenzyme Q reductase was also observed. Both quinones, V and VII, are potent inhibitors of the succinate-coenzyme Q reductase system (Table I) of beef heart submitochondrial particles. This inhibition was reversed by exogenous addition of various concentrations of CoQ_6 to the system. The succinate system is of particular interest in these studies since it is not sensitive to the side chain length of the quinone as shown by Lenaz et al. for the corresponding succinate oxidase system[1]. In contrast to the greater effectiveness of the naphthoquinone, V, in the succinate-cytochrome c reductase system, the quinolinequinone, VII, is more inhibitory than the naphthoquinone in the succinate-coenzyme Q reductase system.

Inhibition of intact human heart mitochondrial succinate-coenzyme Q reductase was observed by quinones, Vi, VI, and VII (Table I). The two quinoline quinones were found to have comparable inhibitory activity and to be more effective inhibitors than the naphthoquinone.

Further studies of the effect of these and other quinone inhibitors in several electron transport systems may further advance the understanding of the function of coenzyme Q in electron transport. This knowledge in turn may facilitate the design of new chemotherapeutic agents based on inhibition of coenzyme Q in the bioenergetic mechanisms of the respiratory chain and coupled phosphorylation.

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