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QUINONE ANALOGUES: A DRUG OF CHOICE FOR THE CONTROL OF FILARIASIS

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Human filariasis is caused by Wuchereria bancrofti, Brugia malayi and B. timori. Of the several recommended model filarial parasites by WHO, Setaria digitata a bovine one has characteristics such as cyanide insensitivity, lack of detectable cytochromes, presence of two quinones Q_8 and Q_6. Of the two quinones Q_8 seems to have a predominant role in energy production. In vitro inhibitory studies using quinone analogues, coenzyme Q_0 and menadione have shown that these compounds paralyse the worms in very low concentrations compared to diethyl carbamazine, the drug of choice for filariasis. The mitochondrial energy production associated with electron transfer is intercepted by quinone analogues. Hence for the treatment of filariasis, this study paves a chemotherapeutic target for the design of drugs which can control the parasites by interacting at the subcellular level by energy depletion.

Filariasis is the most abominable and apparently incurable parasitic disease creating serious public health problems. A global analysis of persons exposed to it has increased from 300 million in 1974 (1) to 900 million in 1989 (2). Filarial control solely depends on earlier detection, chemotherapy and vector control (3). Knowledge about the biochemical differences between the parasites and hosts especially in metabolism is a prerequisite for the formulation of effective drugs to control the disease by chemotherapy. Due to the non-availability of adults of human filarial parasites, Wuchereria bancrofti, Brugia malayi and B. timori, the World Health Organization (WHO) has recommended several filarial parasites as research models (4,5). Of these Setaria digitata a bovine filarial parasite of Bos indicus is reported to be similar to W. bancrofti (5).

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Parasitic helminths are usually oxygen conformers, produce hydrogen peroxide (H_2O_2) during oxygen consumption, possess multiple terminal oxidases and are cyanide insensitive (6). S. digitata is devoid of detectable cytochromes, cyanide insensitive and possesses a rare characteristic, the presence of two quinones Q_6 and Q_8 (7,8). Studies on mitochondrial H_2O_2 and ATP production in this parasite, using different substrates and electron transport complex inhibitors has shown the occurrence of a branched electron transport system in which the quinone Q_8 has a central role (9, 10). Seeking the help of quinone site as a specific target of attack in developing drugs against filariasis, the role of quinone analogues (coenzyme Q_o and menadione) in energy production by electron transfer is envisaged.

Materials and Methods

The local abattoir served as a source for S.digitata and the latter were collected in Tyrode medium (7). The host materials sticking to the surface of the parasite were removed by thorough washing with the medium and kept in it at 37°C until use.

The in vitro effect of quinone analogues (coenzyme Q_o and menadione) is done by incubating the worms in the presence of these compounds. The mitochondriae like particles (MLP) were separated from live worms by differential centrifugation after homogenization in 0.25 M sucrose (10ml/gm wet wt) (7). The amount of H_2O_2 generated was estimated by measuring the decrease in fluorescence of scopoletin dye in the presence of horse radish peroxidase in a Hitachi spectrofluorometer (11) in presence and absence of quinone analogues. The mitochondrial ATP production was done by the method of Williamson and Corkey in a Schimadzu UV 240 spectrophotometer (12). Oxygen uptake by mitochondria was studied using a Gilson oxygraph with Clark type oxygen electrode having a cell capacity of 1.4 ml (13). Protein estimation was carried out by Folin's method (14).

All biochemicals used were purchased from Sigma Chemical Company USA.

Results

The time required for the paralysis of worms in the presence of Q analogues is concentration dependent. Table 1 showed the amount of coenzyme Q_o and menadione necessary for paralysing the worms in vitro in four hours. Figure 1 shows the effect of menadione and coenzyme Q_o on the mitochondrial

Table 1. Concentration of Q analogues and DEC(15) required in *in vitro* to effect paralysis in 4 hr incubation

Compound	Concn. (mM)
Menadione	0.015
Coenzyme Q_o	0.050
DEC	0.70

production of H_2O_2 and ATP using succinate as substrate. Fumarate and malate also behaved in a similar manner. Coenzyme Q_o inhibited the oxygen absorption completely at a concentration of 35μM.

Discussion

The *in vitro* incubation of worms with quinone analogues (Table 1) paralysed the worms even in relatively very low concentrations compared to Diethylcarbamazine (DEC), the drug of choice for filariasis (0.7 mM) (15).

Earlier studies conducted using different substrates (malate, fumarate, succinate and α-glycerophosphate) in

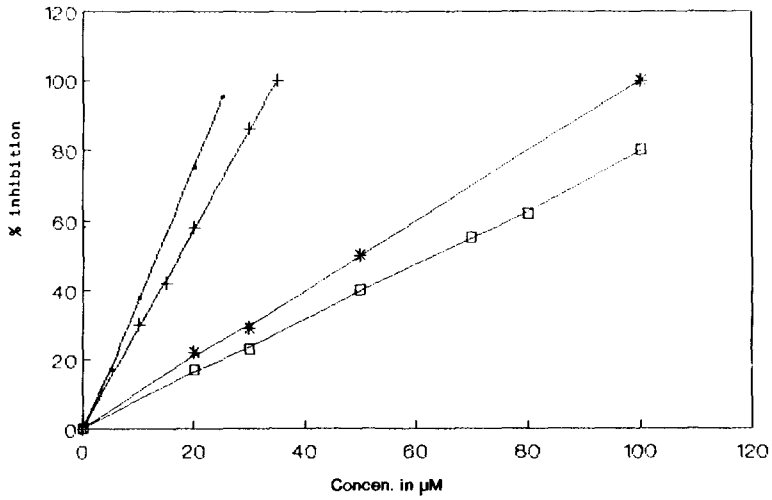

Fig.1 Percentage inhibition of Q_o and menadione on mitochondrial H_2O_2 and ATP production.
—*— Effect of Q_o on H_2O_2 production; —|— Effect of Q_o on ATP production; —✱— Effect of menadione on ATP production; —□— Effect of menadione on H_2O_2 production.

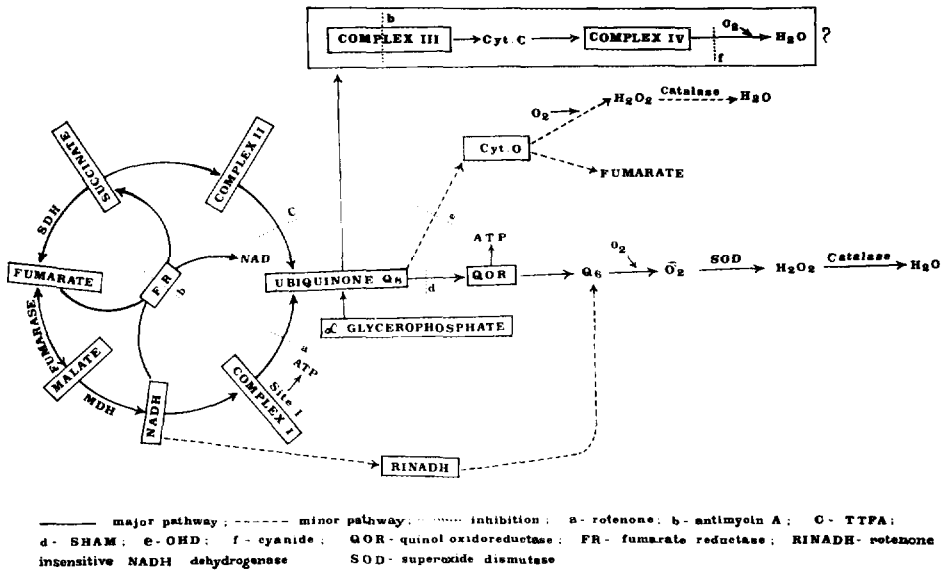

Fig.2 Proposed oxidative electron transport system in S. digitata.

presence and absence of electron transfer complex inhibitors (rotenone, antimycin A, 2 thenoyl trifluoroacetone, o-hydroxydiphenyl, salicylhydroxamic acid etc) on mitochondrial H_2O_2 and ATP production suggested the presence of a branched electron transport system (Fig.2) (9,10). It is clear from the model, that from all substrates the electrons are transferred to quinone Q_8 and then branching occurred to a component analogous to cytochrome o and Q_6. Autooxidation of cytochrome o like component and Q_6 produce H_2O_2 which is detoxified by the presence of calatase.

The production of H_2O_2 and ATP is inhibited by Q_o and menadione which may be due to the blocking of electron transfer. The electrons from the different substrates are accepted by Q_o/menadione instead of Q_8. This prevents further passage of electrons, leading to cessation of oxygen uptake. As the flow of electrons from substrates to Q_o/menadione is effected, the main site of ATP production -Complex I- becomes incapable of coupling ATP due to interception of electrons. Studies from our laboratory also showed that Q_o/menadione is a good acceptor of electrons for the NADH dehydrogenase assay (16). In vitro incubation of E.coli with plumbagin and other redox-active quinones showed that by displacing quinones these compounds

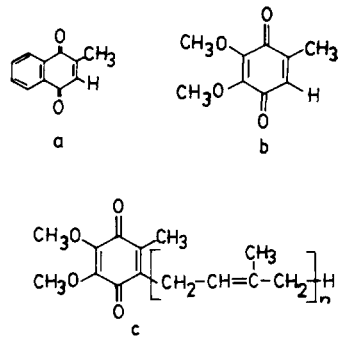

Fig.3 Chemical structure of menadione (a) coenzyme Q_o(b) and quinone (c). For Q_6 n = 6 and for Q_8 n = 8.

intercept electrons from the energy-producing regions of the respiratory chain which leads to cell death (17).

Comparing menadione, coenzyme Q_o is more active in inducing energy depletion. About 30μM of coenzyme Q_o completely inhibited the H_2O_2 production, ATP synthesis and oxygen uptake whereas 100μM of menadione is required to achieve the same level of inhibition. The inhibition seems to be due to their structural analogy to quinones (Fig.3). The antifilarial activity of certain quinolene-containing compounds [chloroquine, (bis) desethyl chloroquine, SN 6911, SN 12108, CN-2999-2k, primaquine, quinacrine and quinine] are reported (18), but their mechanism of action is not known. Based on our studies, we suggest that the antifilarial activity of these drugs may be due to the interception of electron transfer by these quinone analogues.

Complete inhibition of electron transport in most helminths was proposed to require either a specific inhibitor of the central rhodoquinone/b complex or several selective compounds each acting on a different branch of the chain (6). Our studies showed that the quinone analogues used were capable of blocking the electron transfer to the central ubiquinone. The use of hydroxynaphthoquinones and quinolones against malarial parasites may be due to the presence of ubiquinone 8 as a part of respiratory chain, where oxygen uptake and electron transport is disrupted by the hydroxynaphthoquinones (19).

The present study is expected to pave the way for the development of specific drugs for the treatment of filariasis by

the control of parasites using quinone analogues interacting at the subcellular level, inactivating the parasite by energy depletion.

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