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Quinone Analogue Irrecoverably Paralyses the Filarial Parasites *in Vitro*

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2,3-Dimethoxy-5-methyl-1,4-benzoquinone (Q_0), an analogue of ubiquinone, irreversibly paralyses the adult and microfilariae of the cattle filarial parasite *Setaria digitata*. The same concentration of Q_0 that paralyses the microfilariae of *S. digitata* also paralyses the microfilariae of the human filarial parasite *Wuchereria bancrofti* within the same duration. Thus the experiments done in the model *S. digitata* system can well be extended to the human filarial system. A drug at the level of the quinone-centered energy generating system, perhaps an analogue of quinone like Q_0, can inactivate the filarial parasites and may prove to be an effective drug to control filariasis.

Filariasis afflicts millions of people in the tropical and subtropical countries [1, 2]. The question of how to cure this disease still remains unanswered. 1-Diethylcarbamoyl-4-methylpiperazine (DEC) introduced in 1947 [3] is the drug still recommended widely for filariasis [4]. Suramin (antrypol), levamisole, and mebendazole, the DEC analogue centperazine and ivermectin are also suggested for filarial treatment [5–8]. But all of them show severe toxic side effects [6, 9–20]. So it is widely accepted that there is the need for an alternative drug for the treatment of filariasis. The present study is focused on the effect of quinone analogue on the model system, the cattle filarial parasite *Setaria digitata* and also on the microfilaria of both *Setaria* and the human filarial parasite *Wuchereria bancrofti*.

In parasitic helminths it is widely accepted that their specialized electron transport systems are anaerobic in nature even though they possess relatively lower activities of aerobic electron transport system comparable to those of obligate aerobes [21]. The electron transport system of *S. digitata*, the filarial parasite of cattle *Bos indicus*, recommended as a model system for human filarial parasites [10], is branched and possesses rotenone sensitive and rotenone insensitive pathways for NADH oxidation and aerobic and anaerobic pathways for substrate utilization [22] and is characterized by, the presence of two quinones, Q_8 and Q_6, transhydrogenases and fumarate reductase, the absence of typical cytochromes and pyruvate dehydrogenase complex [23–26] and the presence of a mitochondrial quinone-linked lactate utilizing system [27] insensitive to DEC (unpublished).

MATERIALS AND METHODS

S. digitata located in the peritonial cavity of the cattle was collected in Tyrode medium (NaCl 0.8%, KCl 0.02%, $CaCl_2$ 0.02%, $MgCl_2$ 0.01%, $NaHCO_3$ 0.015%, Na_2HPO_4 0.05% and glucose 0.5%) from the local abattoir. The worms freed from extraneous materials were used. They were kept in Tyrode medium at 37°C in a water bath until use. The adult female worms were selected for experimental purposes. The selected worms were of same size and gave similar wriggling score in Tyrode medium. The peristaltic-type wriggling movement of the parasite of curvature from one side to the other of the median and back was scored as one and counts were made for one minute on each organism at the periods indicated. The substrate used were dissolved separately in known volume of Tyrode medium without glucose and its pH adjusted to 7. Then added to different beakers containing Tyrode medium without glucose (final volume 25 ml) and maintained at 37°C. Active female worms were introduced one each into different medii and every 30 minutes interval, number of wriggling was recorded. The quinone analogue, 2,3-dimethoxy-5-methyl-1,4-benzoquinone (Q_0), was dissolved in known volume of Tyrode medium without glucose and was added to beakers containing Tyrode medium without glucose but with substrates and Tyrode medium with glucose. Final volume in each case was 25 ml. Active female worms were introduced one each into these Q_0 containing media of pH 7 maintained at 37°C. Experiments were repeated and simultaneously a control without Q_0 was kept for each test.

The *S. digitata* microfilariae were collected by centrifuging the Tyrode medium in which the female worms were incubated for 2 hrs at 37°C. *W. bancrofti* microfilariae were isolated from heparinised blood samples of filarial patients (collected during night time) by microfiltration. The isolated microfilariae were maintained in Tyrode medium at 37°C. The Q_0 solution prepared in Tyrode medium was added to the microfilaria containing Tyrode medium (final volume 1 ml, pH 7). A control without Q_0 was also maintained. The motility of

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TABLE 1

In Vitro Effect of Q_0 on the Wriggling Movement of *Setaria digitata* in Different Systems

System	% Wriggling (at 30th minute)	Paralysis
a. Tyrode medium without glucose + Q_0 (0.16 mM)	0	(+)
b. a + Glucose	0	(+)
c. a + Malate + NAD + ADP + Pi	0	(+)
d. a + Fumarate	0	(+)
e. a + Sodium lactate	0	(+)
Controls (System minus Q_0)	100	(−)

Note. $N = 6$. Q_0 is 2,3-dimethoxy-5-methyl-1,4-benzoquinone. (+) Gradual and complete paralysis within 30 minutes. (−) No paralysis. 2.5 mM glucose, 0.75 mM NAD. 3 mM each of malate, fumarate, sodium lactate, and, 1 mM each of ADP and P_1 were used.

the microfilaria was observed under a microscope (100×). Different concentrations of Q_0 was tried and a concentration capable of paralysing the worms/microfilariae in a stipulated time was recorded.

RESULTS AND DISCUSSIONS

Q_0, paralyses the worms which were incubated in medium containing different substrates (Table 1). The paralysed worms were found intact and no external damage was noticed. This indicates that the effect of Q_0 may be at the respiratory chain level. The interception of electron transport activity and consequent depletion of energy may lead to the paralysis of worms. *In vitro* incubation of *Escherichia coli* with quinone analogues showed that by displacing endogenous quinones, these analogues intercept and divert

TABLE 2

Recovery Experiments with Q_0-Paralysed Worms

System	Observation (at 2nd hr)
a. PW in Tyrode without glucose	NR
b. a + Glucose	NR
c. a + Fumarate	NR
d. a + Sodium lactate	NR
e. a + Malate + NAD	NR

Note. $N = 6$; NR, No recovery; PW, Q_0-paralysed worms. 2.5 mM glucose, 0.75 mM NAD, and 3 mM each of malate, fumarate, and sodium lactate were used.

electron flow from the energy producing regions of the respiratory chain which leads to cell death [28]. The electron transport in malarial parasite, where Q_8 is the part of the respiratory chain is disrupted by hydroxynaphthoquinolines [29]. 4-hydroxynaphthoquinolines inhibits the electron transport activity in *Elmeria* [30].

S. digitata incubated in Tyrode medium containing glucose was also completely paralysed in presence of Q_0 (Table 1). The inhibition by Q_0 is continuous and irreversible as is clear from the fact that the worms did not recover in Q_0-free medium, after paralysis from exposure to Q_0 (Table 2). The paralysed worms did not recover even when they were incubated in Tyrode medium containing malate plus NAD/sodium lactate/ fumarate (Table 2). Thus from this set of experiments it may be reasonable to conclude that Q_0 irrecoverably affects both aerobic and anaerobic energy generating activities (Figure 1).

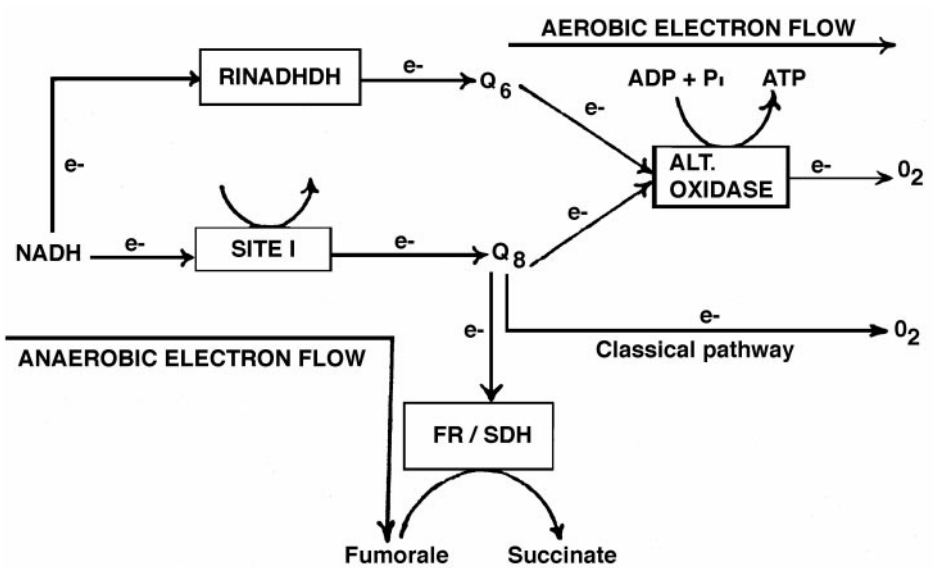

FIGURE 1

TABLE 3

In Vitro Effect of Q_0 on Microfilariae

Microfilaria (mf)	Q_0 concn. (mM)	Paralysis	Recovery
S. digitata mf	0.03	(+)	NR
W. bancrofti mf	0.03	(+)	NR
Controls	0.00	(−)	

Note. (+) Total paralysis within 10 minutes. (−) No paralysis. NR, No recovery. Q_0 is 2,3-dimethoxy-5-methyl-1,4-benzoquinone.

The movement of microfilariae of *S. digitata* is totally arrested within 10 minutes of incubation in Q_0 containing medium (Table 3). The same concentration of Q_0 totally paralyses the microfilariae of the human filarial parasite *W. bancrofti* within the same duration (Table 3). This reveals that the experiments done, with the model *S. digitata* can well be extended to the human filarial system in the direction of drug development. Based on the above observations it can be suggested that a drug at the level of the quinone-centered energy generating system, may be an analogue of quinone like Q_0, can inactivate human filarial parasites and thereby effectively control filariasis.

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