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Interaction of benzoquinones with mitochondria interferes with oxidative phosphorylation characteristics

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Studies with four benzoquinones, viz. juglone, embelin, maesaquinone and maesanin, on rat liver mitochondria oxidative phosphorylation have been carried out. Three of the benzoquinones are uncouplers in the order juglone > maesoquinone > embelin, while maesanin is an inhibitor of electron transport and oxidative phosphorylation.

Benzoquinone; Mitochondria; Oxidative phosphorylation

1. INTRODUCTION

Some benzoquinones occur naturally in the plant family Myrsinaceae. Their pharmacological potential has been known since 1932 when Gokhale and Paranjpe [1] observed that embelin isolated from dried fruits of *Embelia ribes* and *Embelia robusta* was effective against tapeworm. Kawamura and Hokoku [2], using rapanone in later studies, confirmed these findings. Since then, other pharmacological activities have been attributed to specific plant benzoquinones. Maesaquinone, embelin and rapanone or their derivatives have been found to be antibacterial, antifungal or antiamoebic [3–6]. Recent studies indicate that embelin has potential as a fertility regulatory in both males [7–9] and females [10–12].

Eventual use of any drug depends on its toxicological test results. Some studies have indicated that benzoquinones may interfere with respiration. Gupta et al. [13] observed that embelin, rapanone, maesaquinone, dihydromaesaquinone and helicobasidin inhibited respiration in germinating cowpea. Earlier on, Ozawa and Mamose [14] in 1968, reported that alkyl dehydrobenzoquinones caused swelling of the rat liver mitochondria. In the present study, we have investigated the effect of some biologically active benzoquinones on mitochondrial oxidative phosphorylation characteristics. The benzoquinones were: embelin, maesaquinone, maesanin and juglone.

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2. MATERIALS AND METHODS

2.1. *Benzoquinones*

Juglone was purchased from Aldrich Chemical Company, Dorset, UK. Embelin was extracted from *Rapanea melanphloes*; maesaquinone and maesanin from *Maesa lauceolata*. Purification and characterization were performed in the Chemistry Department of the University of Nairobi as described by Midiwo et al. [15].

2.2. *Preparation of rat liver mitochondria*

Rat liver mitochondria were prepared from Sprague–Dawley strains. Mitochondria were isolated using 0.25 M sucrose in presence of 0.5 mg/ml BSA (fatty acid free). The procedure used was essentially as described in [16]. All preparations were carried out at 4°C.

2.3. *Determination of oxygen consumption*

The oxygen consumption rate was recorded polarographically by an oxygen electrode operated at 25°C in a closed and magnetically stirred glass chamber. The reaction medium contained 210 mM mannitol, 70 mM sucrose, 20 mM Tris-HCl buffer, pH 7.4, 3 mM phosphate and 1 mg/ml mitochondrial protein in a total volume of 1.70 ml. The substrate used was either 5 mM K-malate plus 5 mM K-glutamate or 5 mM succinate; in the latter case 5 μM rotenone was included in the reaction mixture. When used, the concentrations of other reagents were as follows: 294 μM ADP, 3 μM CCCP; benzoquinones (in ethanol solution) were added at a concentration of 10 μg/ml.

3. RESULTS AND DISCUSSION

In the absence of benzoquinones (control), the respiratory control ratio (RCR) was 4.72 and 4.64 using malate plus glutamate, and succinate respectively. The ADP/O ratio using malate plus glutamate as the substrate was 2.83 and 1.87 when succinate was used (Fig. 1, trace B and Fig. 2, trace C). This observation indicated that the mitochondria were actively respiring and could effectively phosphorylate ADP to ATP.

Addition of 10 μg/ml of either maesaquinone or embelin (Fig. 1, traces C and D and Fig. 2, traces B and D) resulted in an increase in the rate of state 4 oxygen

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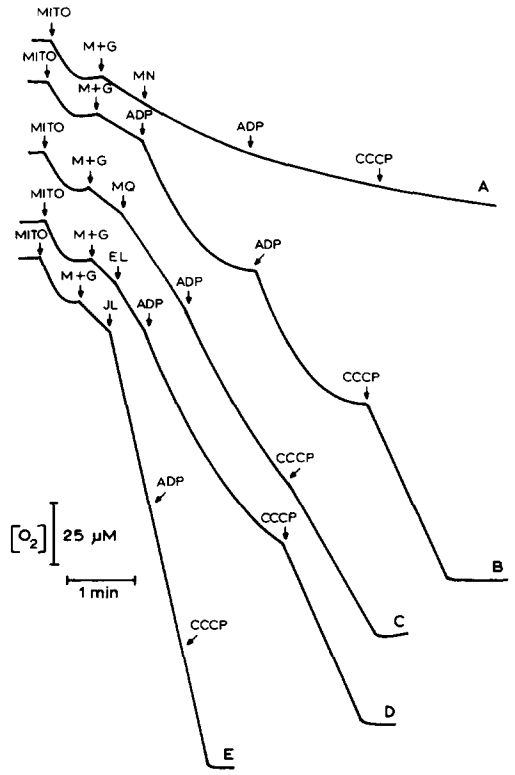

Fig. 1. Oxygen electrode recordings of rat liver mitochondria respiring in a reaction medium consisting of 210 mM mannitol, 70 mM sucrose, 20 mM Tris-HCl, pH 7.4, 0.5 mg/ml BSA and 3 mM phosphate (pH 7.4). When indicated, 1 mg/ml mitochondrial protein (MITO), 294 μM ADP, 3 μM carbonylcyanide-*m*-chlorophenylhydrazone and 10 μM of each of the following: maesanin (MN), maesaquinone (MQ), embelin (EL) and juglone (JL) were added. Reaction chamber volume was 1.70 ml and temperature 25°C.

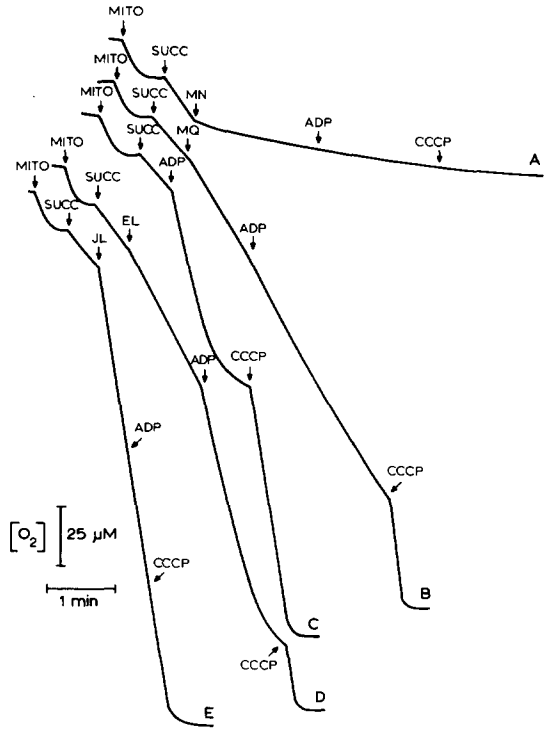

Fig. 2. Oxygen electrode recordings of rat liver mitochondria respiring in a reaction medium consisting of 210 mM mannitol, 70 mM sucrose, 20 mM Tris-HCl, pH 7.4, 0.5 mg/ml BSA and 3 mM phopshate, pH 7.4. When indicated, 1 mg/ml mitochondrial protein (MITO), 5 mM succinate (SUCC) + 5 μM rotenone were added. Other additions were as for Fig. 1.

consumption indicating partial uncoupling of the mitochondria. Subsequent addition of ADP after maesaquinone or embelin resulted in a greater increase in the rate of oxygen consumption compared to that observed after the addition of the two benzoquinones, implying that maesaquinone and embelin do not inhibit ADP consumption. However, compared to the controls, the state 4 rate of oxygen consumption after ADP addition in the presence of the two hydrobenzoquinones was comparatively higher and the RCR was subsequently lower (RCR was 2.17 with embelin and 1.43 with maesaquinone). Subsequent addition of the uncoupler (CCCP after ADP utilisation resulted in a burst in the rate of oxygen consumption indicating that maesaquinone and embelin only partially uncoupled the mitochondria.

The addition of 10 μg/ml juglone to the reaction mixture immediately after adding the substrates resulted in a complete uncoupling of the mitochondria (Fig. 1 and 2, traces E). Subsequent addition of ADP and CCCP after juglone did not accelerate the rate of oxygen consumption as observed with maesaquinone and embelin. This further confirms the fact that juglone completely uncoupled the mitochondria.

From these observations, it is evident that the three benzoquinones are uncouplers of oxidative phosphorylation. The uncoupling potency is in the order juglone > maesaquinone > embelin. Possible mechanisms by which these compounds may uncouple include (a) increasing the mitochondrial membrane leakage to protons as is the case with dinitrophenol [17,18] and/or (b) by providing an alternative route of electron transfer which bypasses much of the electron transport chain so that protons are not translocated as proposed for quinones such as menadione [19]. This uncoupling effect may explain the earlier observations that benzoquinones caused the swelling of rat liver mitochondria [14] as well as inhibition of respiration in germinating cowpea [13].

In both Fig. 1 and 2, traces A, addition of maesanin after the substrates inhibited both the oxygen consumption and subsequent oxidative phosphorylation of ADP. The observation that further addition of the uncoupler CCCP did not relieve the inhibition of oxygen consumption due to maesanin points to a possibility that this benzoquinone may have inhibited the electron transport [20–23]. However, since this inhibition was observed both with malate plus glutamate as well as

succinate plus rotenone it is most likely that maesanin inhibits the electron transport at a point beyond the rotenone site.

Further work on these benzoquinones is suggested to clearly elucidate their mechanism of action.

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