Influence of ubiquinone on the inhibitory effect of adriamycin on mitochondrial oxidative phosphorylation

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1. The inhibition of succinate oxidation in both heart and liver mitochondria by the cardiotoxic anticancer antibiotic adriamycin *in vitro* was reversed to a large extent by exogenous ubiquinone-45. Inhibition of the oxidation of NAD⁺-linked substrates in heart and liver mitochondria responded differently to ubiquinone, the inhibition being reversed only in liver organelles. 2. Administration of adriamycin inhibited oxidative phosphorylation in rat heart, kidney and liver mitochondria, the inhibition being highest in the heart organelles (about 50% for both NAD⁺-linked substrates and succinate). Exogenous addition of ubiquinone to mitochondria isolated from drug-treated animals did not reverse the inhibition. 3. Administration of ubiquinone along with adriamycin did not change effectively the pattern of drug-mediated decrease in oxidative activity of the organelles, particularly in the heart.

Even though the anthracycline antibiotic adriamycin ranks among the most potent anticancer drugs known at present, its therapeutic use is severely restricted by its extreme toxicity, particularly to heart muscles. Severe cumulative dose-dependent cardiomyopathy, leading to congestive heart failure, is the most pronounced toxic side-effect. Drug-induced structural and functional damage to mitochondria is believed to be responsible for myocyte mortality (Ghione & Bertazzoli, 1977). The relation between functional injury to mitochondria and cardiomyopathy was underscored by the observation that adriamycin inhibited mitochondrial oxidation (Gosalvez *et al.*, 1974; Mailer & Petering, 1976; Ferrero *et al.*, 1976; Goormaghtigh *et al.*, 1982). A biochemical explanation for the selective susceptibility of heart tissue to the toxic action of the drug was provided by our observation that succinate oxidation by phosphorylating heart-muscle mitochondria is rendered tremendously sensitive to inhibition by adriamycin by the enzyme hexokinase (Muhammed *et al.*, 1982*a,b*). Tumour cells are characterized by large increases in hexokinase activity, more than 70% of which is associated with the mitochondrial fraction (Burstamante & Pedersen, 1977; Burstamante *et al.*, 1981). We previously reported that in drug-treated animals one-quarter of the amount of drug present in tissues is associated with the mitochondrial fraction (Muhammed & Kurup, 1983).

The structural similarity of adriamycin to ubiquinone, and the important role the lipid quinone plays in mitochondrial function as an oxidation–reduction component of the respiratory chain, have prompted investigators to explore whether administration of ubiquinone would afford relief from cardiotoxicity. Folkers *et al.* (1977) reported that the inhibition of NADH oxidase and succinate oxidase activities of bovine heart mitochondria by anthracycline quinones could be reversed by ubiquinone analogues. Administration of adriamycin to rabbits has been shown to suppress the succinate:ubiquinone reductase activity of heart mitochondria (Bertazzoli *et al.*, 1976). The antibiotic was also shown to inhibit the synthesis of ubiquinone *in vitro* (Folkers *et al.*, 1977). These observations suggested that the antibiotic might act as an antagonist of ubiquinone.

It has been claimed by many research groups that ubiquinone could alleviate symptoms of cardiotoxicity produced by adriamycin. Thus Ghione & Bertazzoli (1977) reported that administration of the lipid quinone to rabbits chronically treated with the antibiotic corrected the abnormalities in electrocardiogram patterns, haematology and some aspects of blood chemistry. In perfused rabbit and rat hearts, exogenous supply of ubiquinone was reported to reverse rapidly the toxic effects of the antibiotic (Bertazzoli & Ghione, 1977; Ohhara *et al.*, 1981). In this context it may be

pertinent that the mitochondria of human and rodent neoplastic tissue have been reported to contain only half the amount of ubiquinone normally present in mitochondria (Kishi *et al.*, 1977).

In view of the above reports, which highlight the potential of ubiquinone as an agent for the amelioration of the toxic effects of adriamycin, we have investigated the effect of ubiquinone on the inhibitory action of the antibiotic on mitochondrial oxidative phosphorylation. The results are presented and their implications discussed in this paper.

Materials and methods

Animals and administration of drugs

Male Wistar rats (3 months old), weighing 150 g and obtained from the Central Animal Facility of this Institute, were fed with Commercial Hind Lever diet, and used in the experiments reported here. Experimental animals were injected with adriamycin (0.5 mg in 0.2 ml of water/rat per day) intraperitoneally for 8 days, and with ubiquinone (0.5 mg/rat per day) in 0.2 ml of peanut oil for the same period. Control animals were fed with 0.2 ml of peanut oil daily. In the experiments reported here only ubiquinone-45 was used.

Determination of oxidative phosphorylation

The animals were killed by cervical dislocation, and mitochondria from liver, heart and kidney were isolated as described by Muhammed *et al.* (1982b). To minimize variations, an equal number of control and drug-treated animals were killed at about 09:00 h.

Oxidative phosphorylation was determined both manometrically and polarographically, as described previously (Muhammed *et al.*, 1982b).

Protein was measured by the biuret method, deoxycholate being used for solubilization (Gornall *et al.*, 1949). All solutions were prepared in water, double-distilled in an all-quartz apparatus. Solutions were adjusted to pH 7.4 before use.

Results

Effect of ubiquinone in vitro

We have shown that, under phosphorylating conditions, substrate oxidation by heart, kidney and liver mitochondria is inhibited by adriamycin *in vitro*, the concentration for 50% inhibition being in the range 0.5–1 mM. However, in the presence of the enzyme hexokinase, succinate oxidation by heart mitochondria becomes highly sensitive to inhibition by the drug, 50% inhibition being given by 5 μ M-adriamycin (Muhammed *et al.*, 1982a,b). The effect of addition of ubiquinone on the inhibitory

activity of adriamycin was tested with concentrations of the antibiotic that gave about 50% inhibition of oxygen uptake. The results are summarized in Table 1.

With both heart and liver mitochondria, the inhibition by adriamycin of oxygen uptake was reversed to a large extent (85%) by addition of ubiquinone when succinate was the substrate. These results are in agreement with the studies of Kishi *et al.* (1977) with bovine heart mitochondria and ubiquinone-50. Similar experiments on the oxidation of NAD⁺-linked substrates yielded opposite effects with the two mitochondrial preparations. With both liver and heart mitochondria, oxidation was drastically suppressed by the addition of exogenous ubiquinone (Table 1). However, with liver mitochondria, ubiquinone could reverse (by 85%) the inhibition by the antibiotic. In contrast, ubiquinone did not reverse the inhibition in heart mitochondria (Table 1). The inhibition of NADH oxidation by exogenous ubiquinone in bovine heart mitochondria has been noted by Kishi *et al.* (1977), who, however, obtained a 50% reversal of the inhibition by adriamycin.

Effect of adriamycin in vivo

Consistent with our observations that adriamycin was a potent inhibitor of mitochondrial oxidative phosphorylation (Muhammed *et al.*, 1982a,b) and that mitochondria in heart, liver and kidney accumulated the drug (Muhammed & Kurup, 1983), the organelles isolated from the organs of adriamycin-treated animals showed decreased oxidative phosphorylation (Table 2).

In kidney mitochondria, NAD⁺-linked oxidation was suppressed by 37% and succinate oxidation by 27%. Liver mitochondria were less susceptible, the inhibition being only about 20%. The inhibition was highest in heart mitochondria, NAD⁺-linked oxidation being depressed by 51% and succinate oxidation by 47%. The mitochondrial samples from kidney, liver and heart contained 220, 144 and 132 pmol of the drug/mg of protein respectively (Muhammed & Kurup, 1983). The greater susceptibility of heart mitochondria to the inhibitory action of the drug and its metabolites is evident.

The results in Table 2 indicated that treatment with adriamycin did not affect the phosphorylation capacity of mitochondria; the P/O ratio did not show any change. However, *in vitro* the drug showed uncoupling properties (Muhammed *et al.*, 1982b). In the manometric method, the large excess of hexokinase present would tend to minimize the uncoupling action of the drug by making ATP limiting and thus unavailable for hydrolysis (Slater, 1966). To eliminate this possibility, oxidative phosphorylation was measured

Table 1. *Effect of exogenous ubiquinone on the inhibition of mitochondrial oxidation by adriamycin in vitro*

The rate of oxidation was determined manometrically by using the 'hexokinase trap'. A 3 ml reaction system contained about 1 mg of mitochondrial protein. Ubiquinone-45 (0.5 mM) was added as an emulsion in water as indicated. Heart mitochondria received 0.5 mM-adriamycin when pyruvate + malate was the substrate and 30 μ M-adriamycin when succinate was the substrate. Liver mitochondria received 1 mM-adriamycin and 1 mM-ubiquinone-45. Values are means $\pm$ S.D. for three independent determinations (samples of mitochondria).

Substrate	Addition	Oxygen uptake (ng-atoms of O/min per mg of protein)	
		Heart	Liver
Pyruvate + malate	None	131 $\pm$ 6	86 $\pm$ 7
	Ubiquinone	94 $\pm$ 18	42 $\pm$ 5
	Adriamycin	46 $\pm$ 12	43 $\pm$ 1
	Ubiquinone + adriamycin	41 $\pm$ 8	74 $\pm$ 1
Succinate	None	201 $\pm$ 17	162 $\pm$ 7
	Ubiquinone	207 $\pm$ 40	167 $\pm$ 9
	Adriamycin	98 $\pm$ 7	46 $\pm$ 10
	Ubiquinone + adriamycin	169 $\pm$ 26	140 $\pm$ 19

Table 2. *Effect of administration of adriamycin on oxidative phosphorylation by rat heart, liver and kidney mitochondria*

Rats were given adriamycin (0.5 mg/rat per day) intraperitoneally for 8 days. Oxidative phosphorylation was determined by manometry by using the 'hexokinase trap'. Oxygen-uptake values (ng-atoms of O/min per mg of mitochondrial protein) are means $\pm$ S.D. for eight to ten independent determinations (animals). ** P < 0.01.

Substrate	Treatment	Heart		Liver		Kidney	
		Oxygen uptake	P/O ratio	Oxygen uptake	P/O ratio	Oxygen uptake	P/O ratio
Pyruvate or glutamate + malate	None	140 $\pm$ 27	2.5 $\pm$ 0.4	95 $\pm$ 11	2.4 $\pm$ 0.1	76 $\pm$ 7	2.4 $\pm$ 0.4
	Adriamycin	68 $\pm$ 19**	2.5 $\pm$ 0.4	70 $\pm$ 13**	2.4 $\pm$ 0.2	48 $\pm$ 11**	2.3 $\pm$ 0.2
Succinate	None	213 $\pm$ 31	1.4 $\pm$ 0.1	184 $\pm$ 20	1.6 $\pm$ 0.2	205 $\pm$ 19	1.3 $\pm$ 0.2
	Adriamycin	113 $\pm$ 23**	1.5 $\pm$ 0.3	152 $\pm$ 13**	1.5 $\pm$ 0.2	149 $\pm$ 18**	1.2 $\pm$ 0.3

Table 3. *Effect of administration of adriamycin on oxidation and respiratory control index of rat heart and liver mitochondria*

Animals were given adriamycin (0.5 mg/rat per day) for 8 days. Oxidative phosphorylation was determined by the polarographic method. 'State 3' (ADP added) and 'State 4' (ADP exhausted; see Chance & Williams, 1955) rates of oxidation are expressed as ng-atoms of O/min per mg of protein. Respiratory control index (RCI) is calculated as the ratio of oxygen-uptake rates in State 3 and State 4. Values are means $\pm$ S.D. for four independent determinations.

* P < 0.05; ** P < 0.01.

Substrate	Treatment	Heart	Liver		
		State 3	State 3	State 4	RCI
Pyruvate + malate	None	142 $\pm$ 25	73 $\pm$ 4	12 $\pm$ 2	6.4 $\pm$ 0.8
	Adriamycin	85 $\pm$ 15**	60 $\pm$ 5*	15 $\pm$ 2	4.1 $\pm$ 0.7*
Succinate	None	174 $\pm$ 16	202 $\pm$ 36	33 $\pm$ 9	5.6 $\pm$ 0.8
	Adriamycin	113 $\pm$ 6**	156 $\pm$ 25**	35 $\pm$ 10	4.7 $\pm$ 0.6*

by the polarographic method. The results are presented in Table 3.

Heart mitochondria isolated from drug-treated animals showed lowered rates of State-3 oxidation (40%), in agreement with the results obtained by

the manometric method (Table 2). The lowering of the respiratory control of liver mitochondria was not achieved by an increase in State-4 oxidation, but by a decrease of about 25% in the rate of active (State 3) oxidation. This effect is similar to that

Table 4. *Effect of exogenous ubiquinone on substrate oxidation by cardiac and hepatic mitochondria of adriamycin-treated rats*
The reaction system and method of assay were the same as described in the legend of Table 1, except that mitochondria isolated from heart and liver of rats which were given the drug (0.5 mg/rat per day, intraperitoneally) were also used as indicated. Oxygen-uptake values (ng-atoms of O/min per mg of mitochondrial protein) are means $\pm$ S.D. for four independent determinations (animals). Ubiquinone-45 (0.5 mM) was added as an emulsion in water.

Substrate	Addition	Heart		Liver	
		Control	Adriamycin	Control	Adriamycin
Pyruvate or glutamate + malate	None	106 $\pm$ 19	50 $\pm$ 8	102 $\pm$ 16	79 $\pm$ 13
	Ubiquinone	73 $\pm$ 11	43 $\pm$ 16	59 $\pm$ 10	61 $\pm$ 19
Succinate	None	181 $\pm$ 15	92 $\pm$ 13	232 $\pm$ 21	207 $\pm$ 26
	Ubiquinone	138 $\pm$ 25	92 $\pm$ 7	175 $\pm$ 19	159 $\pm$ 23

Table 5. *Effect of administration of ubiquinone and adriamycin on oxidative activity of rat heart and liver mitochondria*
Animals were given adriamycin (0.5 mg/rat per day) intraperitoneally and ubiquinone-45 (0.5 mg/rat per day) orally in peanut oil for 8 days as indicated. Oxidation was measured by the manometric procedure. Values are means $\pm$ S.D. for seven independent determinations for the heart, and means of two determinations for liver mitochondria.
** $P < 0.01$.

Substrate	Treatment	Oxidation (ng-atoms of O min per mg of protein)	
		Heart	Liver
Pyruvate + malate	None	129 $\pm$ 13	72
	Ubiquinone	129 $\pm$ 25	53
	Adriamycin	62 $\pm$ 6**	59
	Ubiquinone + adriamycin	74 $\pm$ 2**	75
Succinate	None	235 $\pm$ 2	216
	Ubiquinone	212 $\pm$ 16	147
	Adriamycin	137 $\pm$ 25	175
	Ubiquinone + adriamycin	139 $\pm$ 23	173

observed in the inhibitory studies *in vitro* reported by Muhammed *et al.* (1982b). Ferrero *et al.* (1976) reported that mitochondria isolated from the hearts of rabbits intermittently treated with adriamycin showed a decrease in respiratory control largely due to a 30% increase in State-4 oxidation.

We decided to see whether the decrease in oxidation in mitochondria isolated from drug-fed animals could be corrected by addition of ubiquinone in the reaction system. The results presented in Table 4 make it abundantly clear that ubiquinone does not reverse the inhibition in either heart or liver mitochondria.

Effect of ubiquinone in vivo

Ubiquinone administered along with adriamycin did not bring about any effective change in the drug-mediated decrease in the oxidative activity of heart mitochondria, irrespective of the type of substrate being oxidized (Table 5). With liver mitochondria also, the results were not encouraging. Administration of ubiquinone itself resulted in a considerable decrease in the oxidative capability of

mitochondria. The small decrease in oxidation brought about by adriamycin administration was not reversed when succinate was being oxidized. The results presented for pyruvate oxidation would indicate that the inhibitory actions of ubiquinone and adriamycin cancel each other, thus resulting in the restoration of oxidation (Table 5). However, as noted above (Table 2), the inhibitory influence of adriamycin on the oxidation of NAD⁺-linked substrates by liver mitochondria is not pronounced.

Discussion

The results presented in this paper, which demonstrate substantial inhibition of the rate of substrate oxidation by mitochondria isolated from the organs of drug-treated animals, are consistent with and explicable on the basis of our previous work (Muhammed *et al.*, 1982a,b; Muhammed & Kurup, 1983). These findings, particularly the higher inhibition of oxidation shown by heart mitochondria despite the concentration of the drug in the organ being no more than in other organs

such as the liver, lend support to the widely held notion that functional damage to mitochondria is a principal factor in the extreme cardiac toxicity of the drug.

The demonstration that the inhibitory action of added adriamycin on the oxidative activity of isolated mitochondria could be reversed to some extent by ubiquinone *in vitro* (Kishi *et al.*, 1977) has kindled hopes of using the lipid quinone as an antidote for the toxicity of the antibiotic. The observation in many laboratories (Ghione & Bertazzoli, 1977; Ohhara *et al.*, 1981) that some symptoms of cardiac toxicity were relieved by administration of ubiquinone added physiological relevance to the inferences made from the studies *in vitro*. Viewed in this light, experiments on the effects of ubiquinone administration on mitochondrial oxidative phosphorylation in drug-treated animals assume importance.

The results presented in this paper clearly show that, when administered together, ubiquinone does not reverse the deleterious effects of adriamycin on the oxidative function of cardiac mitochondria (Table 5). Also, ubiquinone does not stimulate substrate oxidation when added to mitochondria isolated from adriamycin-fed animals. However, when added with adriamycin to normal mitochondria, the lipid quinone could reverse the inhibitory action of the drug on succinate oxidation, which is the most sensitive reaction (Table 1; Muhammed *et al.*, 1982a,b). This raises the question whether exogenously administered ubiquinone permeates the organ. According to Jayaraman *et al.* (1963), ubiquinone administered by any route (oral, intracardial or intraperitoneal) does not penetrate the heart. More recently, Nakamura *et al.* (1980) have claimed that a small fraction (0.07%) of intravenously injected ubiquinone-50 enters rat heart, and about one-fifth of this is transferred to the mitochondrial inner membrane. Evidently the concentration of ubiquinone entering the organelles will be too meagre to be of any physiological significance.

Moreover, entry of the quinone into an organ or organelles may not be the decisive factor for physiological efficacy, because in the liver, which is known to accumulate a major portion of exogenously administered ubiquinone (Jayaraman *et al.*, 1963), reversal of inhibition is confined to NAD⁺-linked substrates (Table 5). It is possible that, to be physiologically functional, the quinone has to be associated with a specific binding protein (Yu & Yu, 1981). It may also be pertinent that, in instances in which amelioration of clinical symptoms of adriamycin toxicity to the heart was shown, prolonged administration of ubiquinone before treatment with the drug was required (Ghione & Bertazzoli, 1977). It appears that the

protective action of ubiquinone rests largely on its antioxidant properties (Yamanaka *et al.*, 1980), is not specific for adriamycin and related drugs, but is shown in conditions such as ischaemia (Katagiri *et al.*, 1981). The ability of the drug to interact with mitochondrial redox systems and to generate free radicals is well documented (Donehower *et al.*, 1979; Davies *et al.*, 1983; Gutierrez *et al.*, 1983). The possibility of ubiquinone interacting with the drug outside the mitochondrial membrane is also indicated by our results.

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