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Bioenergetics in clinical medicine: Prevention by forms of coenzyme Q of the inhibition by adriamycin of coenzyme Q_{10}-enzymes in mitochondria of the myocardium*

(ubiquinone/cardiotoxicity/mitochondrial enzymes/electron transfer)

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ABSTRACT **Adriamycin inhibits the succinoxidase system and the NADH-oxidase system. Both of the intact mitochondrial enzymes and the pentane-extracted preparations are inhibited. The inhibition can be prevented by a molar ratio of coenzyme to adriamycin of 3:1 for coenzyme Q_{10} (ubiquinone), 5:1 for coenzyme Q_7, and 5:1 for coenzyme Q_4. Prevention of inhibition was observed in the decreasing order of coenzyme Q_{10} > coenzyme Q_7 > H_6 coenzyme Q_4 > coenzyme Q_4. Adriamycinone was three times more inhibitory than adriamycin, which is compatible with a less polar fragment necessary to inhibit the lipoidal coenzyme Q_{10}. Daunomycinone was not inhibitory at a concentration at which adriamycinone is effective, indicating that the hydroxyl group of the latter could be binding at the receptor, since it should not influence electron transfer of rings B and C.**

Adriamycin and related anthracyclines are now widely used in clinical medicine for the chemotherapy of cancer (1). Carter and Blum (2) summarized the wide range of clinical antitumor activities and the integration of adriamycin into combined modality protocols. The clinical use of adriamycin is accompanied by serious side-effects, which rigidly restrict its use. Although cardiotoxicity is the most serious side-effect in cancer patients, there is a spectrum of other major side-effects, including hematologic toxicity. The biochemical elucidation of the mechanism (3) of these side-effects, particularly the cardiotoxicity, has become urgent, and particularly if such knowledge could minimize side-effects.

We have considered that the cardiotoxicity of adriamycin might be due to inhibition of coenzyme Q_{10}-enzymes. Data have now been obtained on four forms of coenzyme Q (CoQ) that show that the coenzymes prevent this inhibition *in vitro*. The biochemical basis of this study of cardiotoxicity is the knowledge that coenzyme Q_{10} (ubiquinone) is an indispensable component of the succinoxidase and the NADH-oxidase systems and other CoQ_{10}-enzymes which are essential to the electron transfer mechanism and oxidative phosphorylation ("bioenergetics") of mitochondria of the human myocardium. Inhibition (toxicity) of enzymes of respiration could be damaging to the heart, and could range from a minor to a major component of the cardiotoxicity of adriamycin.

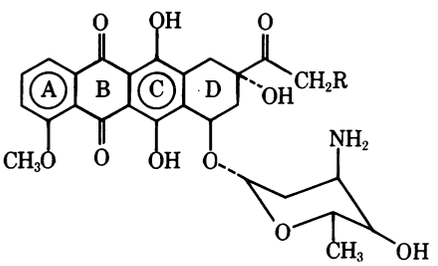
METHODS

The methods for these assays with adriamycin, the two aglycones, and the four forms of coenzyme Q have been reported (4). Preparation of beef heart mitochondria (5) and extraction with pentane (6) were described. The determination of the specific activities of succinoxidase and NADH-oxidase, inhibition by adriamycin (7), and determination of protein were described (8). The antimetabolite CoQ_{10}-enzyme preparations were detailed (9).

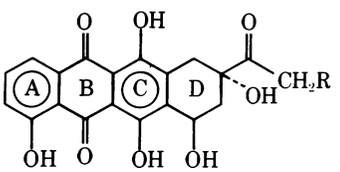
RESULTS

The data (Table 1) show that CoQ_{10} prevents the inhibition by adriamycin (I) of both the CoQ_{10}-enzymes, succinoxidase and NADH-oxidase.

For succinoxidase, it was found that 2.5 μmol per flask of adriamycin caused an inhibition of 46%. Inhibition levels of about 50% were sought. Increasing the levels of CoQ_{10} from 0.5 to 10.0 μmol increased the relative activity to about 100%.



I: R = OH II: R = H



III: R = OH IV: R = H

For NADH-oxidase, adriamycin was about twice as inhibitory as for succinoxidase, since 1.3 μmol resulted in a relative activity of 47%. Increasing the levels of CoQ_{10} from 0.5 to 2.5 μmol increased the relative activity from about 67 to 75%.

It may be considered that the preparations of succinoxidase and NADH-oxidase "simulate" the intact condition of these two enzymes, although these preparations are probably "less intact" than *in situ*. The state of the enzyme as it exists in the mitochondria of the myocardium of a cancer patient receiving adriamycin is important. Succinoxidase and NADH-oxidase are either saturated or unsaturated with CoQ_{10}; unsaturation is equivalent to a deficiency of CoQ_{10}. Many cancer patients, particularly in the older age brackets, may have a deficiency of CoQ_{10} in their myocardium before they are treated with adriamycin. Littarru, Ho, and Folkers (10) showed that about 75% of 123 cardiac patients had a deficiency of CoQ_{10} in heart biopsies. "*In vitro* simulation" of unsaturation with respect to CoQ_{10} in the myocardium may be obtained by solvent extraction for removal of CoQ_{10} (11). Pentane was used for ex-

Abbreviation: CoQ, coenzyme Q.

* This is paper VII of a series. Paper VI is Wilkinson, E. G., Arnold, R. M. & Folkers, K. (1976) *Res. Commun. Chem. Pathol. Pharmacol.* 14, 715–719.

Table 1. Prevention by coenzyme Q_{10} of inhibition by adriamycin of coenzyme Q_{10}-enzymes

CoQ_{10} (μmol/flask)	Succinoxidase* Specific activity	Succinoxidase* Relative activity (%)	NADH-oxidase† Specific activity	NADH-oxidase† Relative activity (%)
Control	0.493	100	0.802	100
0	0.227	46	0.374	47
0.5	0.256	52	0.494	67
2.5	0.261	53	0.605	75
5.0	0.295	60		
10.0	0.513	104		

Each flask contained 2.8 ml of reaction mixture. Specific activity was expressed as μatoms of O_2/mg of protein per min.

* Concentration of adriamycin was 2.5 μmol per flask, and the mitochondrial protein was 0.68 mg, which contained 3.0 nmol of CoQ_{10} per mg of protein.

† Concentration of adriamycin was 1.3 μmol per flask, and the mitochondrial protein was 0.45 mg, which contained 3.4 nmol of CoQ_{10} per mg of protein.

traction; the data on the prevention by CoQ_{10} of inhibition by adriamycin are in Table 2. Succinoxidase and NADH-oxidase, after pentane extraction, had relative activities of 19 and 7%, respectively. Supplementation with 0.04 μmol of CoQ_{10} gave a relative activity of 100% for both enzymes. Increasing the levels of adriamycin from 1.0 to 3.2 μmol in the presence of 0.04 and 0.1 μmol of CoQ_{10} caused the relative activities to decrease from 81 to 19% for succinoxidase and 97 to 56% for NADH-oxidase. For NADH-oxidase, 0.1 μmol of CoQ_{10} was necessary to achieve a relative activity of 100%.

The data in Table 3 show the prevention by CoQ_4, H_6CoQ_4, CoQ_7, and CoQ_{10} of the inhibition of succinoxidase by adriamycin. With 2.0 μmol of adriamycin, 10 μmol of CoQ_7 and of CoQ_{10} prevented the inhibition, with an indication that CoQ_{10} might be superior on a molar basis to CoQ_7. Levels of 10–100 μmol of CoQ_4, 10 μmol of H_6CoQ_4, and 10 μmol of CoQ_7 did not completely prevent the inhibition by 2.5 μmol of adriamycin, but 10 μmol of CoQ_{10} completely prevented the inhibition. A level of 3.0 μmol of adriamycin decreased the relative activity to 21% and 10 and 100 μmol of CoQ_4 and 10 μmol of CoQ_7 partially prevented the inhibition, as shown by relative activities of 40–54%, but 10 μmol of CoQ_{10} completely prevented the inhibition by 3.0 μmol of adriamycin.

These forms of CoQ prevent the inhibition by adriamycin

Table 2. Inhibition by adriamycin of pentane-extracted mitochondrial coenzyme Q_{10}-enzymes

Adriamycin (μmol/flask)	Succinoxidase CoQ_{10} (μmol/flask)	Succinoxidase Relative activity (%)	NADH-oxidase CoQ_{10} (μmol/flask)	NADH-oxidase Relative activity (%)
0	0	19	0	7
0	0.04	100	0.1	100
1.0	0.04	81	0.1	97
2.0	0.04	55	0.1	71
2.4	0.04	25	0.1	72
2.8	0.04	19	0.1	73
3.2	0.04	19	0.1	56

Adriamycin was added to the reaction mixture immediately after the addition of CoQ_{10}. The mixture contained 0.61 mg of pentane-extracted mitochondrial protein.

Table 3. Prevention by forms of coenzyme Q of inhibition by adriamycin of mitochondrial succinoxidase

Additions* (μmol/flask)	Specific activity†	Relative activity (%)
None	0.499	100
Adriamycin (2.0)	0.294	59
+ CoQ_4 (10)	0.529	106
+ CoQ_4 (100)	0.474	95
+ CoQ_7 (10)	0.459	92
+ CoQ_{10} (10)	0.628	126
Adriamycin (2.5)	0.150	30
+ CoQ_4 (10)	0.284	57
+ CoQ_4 (100)	0.309	62
+ H_6CoQ_4 (10)	0.349	70
+ CoQ_7 (10)	0.409	82
+ CoQ_{10} (10)	0.519	104
Adriamycin (3.0)	0.105	21
+ CoQ_4 (10)	0.259	52
+ CoQ_4 (100)	0.200	40
+ CoQ_7 (10)	0.269	54
+ CoQ_{10} (10)	0.544	109

* Adriamycin, m.p. 206° (dec). Number of μmol of adriamycin required for 50% inhibition was 2.13, giving an antimetabolite CoQ index of 906. The CoQ homologues were dissolved in absolute ethanol and then dispersed into the reaction mixture before the mitochondria and adriamycin were added.

† Specific activity was expressed as μatoms of O_2/mg of protein per min. Each flask contained 0.59 mg of mitochondrial protein, with 4.0 nmol of CoQ per mg of protein in 2.8 ml.

in the descending order: $CoQ_{10} > CoQ_7 > H_6CoQ_4 > CoQ_4$, for succinoxidase (Table 4). CoQ_{10}, CoQ_7, and CoQ_4 were reassayed at 10 μmol per flask, for prevention of inhibition by 2.5 μmol per flask of adriamycin. The relative activities were 116 ± 7 for CoQ_{10}, 77 ± 5 for CoQ_7, and 43 ± 8 for CoQ_4. The differences between these activities are significant, $P < 0.01$. Again, CoQ_{10} was superior. However, it seemed that CoQ_7 should completely prevent inhibition at a higher level, and this result is shown as follows.

Table 5 shows that 2.5 and 2.0 μmol of adriamycin reduced the relative activities of succinoxidase from 100% to 25 and 49%, respectively. A level of 10 μmol of CoQ_7 prevented the inhibition by 2.0 μmol of adriamycin, and represents a 5-fold ratio of CoQ_7 to adriamycin. Table 3 shows that a 3-fold excess of CoQ_{10} prevented the inhibition. Increasing the levels of CoQ_7

Table 4. Statistical comparison of prevention by forms of coenzyme Q of inhibition by adriamycin of mitochondrial succinoxidase

Additions (μmol/flask)	Relative activity (%) Mean ± SD	Experiment 1	2	3	4	5
None*	100					
Adriamycin (2.5)	31 ± 2†	30	32	30	29	33
+ CoQ_4 (10)	43 ± 8†	39	41	38	40	57
+ CoQ_7 (10)	77 ± 5†	77	73	71	81	83
+ CoQ_{10} (10)	116 ± 7†	118	117	117	124	104

Mitochondrial protein was 0.72 mg/flask.

* Mean of specific activity for the control was 0.652 ± 0.046 μatom of O_2/mg of protein per min.

† Difference of these respective values was significant, $P < 0.01$.

Table 5. Prevention by coenzyme Q_7 of the inhibition by adriamycin of mitochondrial succinoxidase

Adriamycin* (μmol/flask)	Specific activity†	Relative activity (%)
Experiment 1		
Control	0.641	100
Adriamycin (2.5)	0.158	25
(2.0)	0.312	49
(1.0)	0.692	108
(0.5)	0.662	103
(2.5) + CoQ_7 (10)	0.468	73
(2.0) + CoQ_7 (10)	0.591	92
(1.0) + CoQ_7 (10)	0.709	111
(0.5) + CoQ_7 (10)	0.706	110
Experiment 2		
Control	0.533	100
Adriamycin (2.0)	0.276	52
+ CoQ_7 (10)	0.464	87
+ CoQ_7 (20)	0.472	88
+ CoQ_7 (30)	0.489	92
+ CoQ_7 (40)	0.472	88

Experiment 1: the mitochondrial protein was 0.64 mg per flask. Experiment 2: the mitochondrial protein was 0.73 mg per flask. Each flask contained 2.8 ml of reaction mixture.

* CoQ_7 was preincubated with the mitochondrial preparation prior to addition of adriamycin.

† Specific activity was expressed as μatom of O_2/mg of protein per min.

from 10 to 40 μmol resulted in about 90% relative activity as compared to 50% for 2.0 μmol of adriamycin (Table 5).

The aglycones of adriamycin (I) and daunomycin (II) are adriamycinone (III) and daunomycinone (IV), respectively. Adriamycinone (Table 6) was about three times as potent an inhibitor of succinoxidase as adriamycin; 0.5 μmol of adriamycinone gave about 50% relative activity. This inhibition by adriamycinone of succinoxidase was not prevented as effectively by CoQ_{10} as was the inhibition by adriamycin.

In contrast to adriamycinone, which at 2.5 μmol resulted in a relative activity of 11%, 0.5–2.5 μmol of daunomycinone did not inhibit succinoxidase.

Table 6. Inhibition by adriamycinone and daunomycinone of mitochondrial succinoxidase

Additions (μmol/flask)	Specific activity*	Relative activity (%)
Experiment 1†		
Control	0.878	100
Adriamycin (2.5)	0.239	27
Adriamycinone (2.5)	0.099	11
(1.0)	0.281	32
(0.5)	0.416	47
(0.2)	0.746	85
(0.1)	0.773	88
(2.5) + CoQ_{10} (10)	0.083	9
(1.0) + CoQ_{10} (10)	0.273	31
(0.5) + CoQ_{10} (10)	0.527	60
(0.2) + CoQ_{10} (10)	0.448	51
(0.1) + CoQ_{10} (10)	0.702	80
Experiment 2†		
Control	0.651	100
Adriamycin (2.5)	0.201	31
Adriamycinone (0.5)	0.247	38
+ CoQ_7 (10)	0.240	37
+ CoQ_7 (20)	0.247	38
+ CoQ_7 (30)	0.234	36
+ CoQ_7 (40)	0.241	37
+ CoQ_{10} (10)	0.306	47
+ CoQ_{10} (20)	0.228	35
Experiment 3†		
Control	0.519	100
Adriamycin (2.5)	0.142	27
Daunomycinone (2.5)	0.522	101
(1.0)	0.503	97
(0.5)	0.533	103
(2.5) + CoQ_{10} (10)	0.589	113
(1.0) + CoQ_{10} (10)	0.584	113

The mitochondrial protein was 0.59 mg per flask for Exp. 1, 0.55 mg per flask for Exp. 2, and 0.73 mg per flask for Exp. 3.

* Specific activity was expressed as μatom of O_2/mg of protein per min.

† CoQ was preincubated with the mitochondrial preparation for 10 min prior to the addition of adriamycinone or daunomycinone.

DISCUSSION

Adriamycin is an effective inhibitor of succinoxidase and NADH-oxidase, "intact" mitochondrial enzymes, preparations of which had been extracted with pentane to reduce the level of CoQ_{10}. It was considered that such extracted preparations might "simulate" an unsaturated state with respect to CoQ_{10} in a cardiac deficiency of CoQ_{10}. Some cancer patients might have a myocardial deficiency of CoQ_{10} before treatment with adriamycin, and might show greater cardiotoxicity than a patient with no preexisting cardiac deficiency of CoQ_{10}.

Although CoQ_{10} was more effective than CoQ_7 on a molar basis in preventing inhibition, a higher molar ratio of CoQ_7 to adriamycin was as effective as CoQ_{10}. A 3-fold ratio of CoQ_{10} to adriamycin prevented inhibition, and a 5-fold ratio of CoQ_7 to adriamycin prevented inhibition. H_6CoQ_4 and CoQ_4 were less effective than CoQ_7. Possibly, higher ratios of H_6CoQ_4 and CoQ_4 to adriamycin would effectively prevent inhibition.

These differences, *in vitro*, between CoQ_{10}, CoQ_7, H_6CoQ_4, and CoQ_4 for prevention of the inhibition may not be applicable *in vivo*. The lower-molecular-weight CoQs, such as CoQ_4, H_6CoQ_4, and CoQ_7, might possibly reach these mitochondrial enzymes better than CoQ_{10} (12).

The inhibition by adriamycinone was not as effectively prevented by CoQ_{10} as was the inhibition by adriamycin. Although daunomycinone differs from adriamycinone by only one hydroxyl group, daunomycinone was less inhibitory to succinoxidase than was adriamycinone. Daunomycinone at 2.5 μmol did not inhibit; adriamycinone gave about 90% inhibition. The antimetabolite CoQ_{10}-index of adriamycin is 906 (4) and that of adriamycinone is 194, calculated from Table 6. The absence of a single hydroxyl group may cause daunomycinone to be unfavorable for the receptor of succinoxidase for CoQ_{10} and adriamycinone. This hydroxyl group could influence binding of adriamycinone at the receptor since it may not influence electron transfer.

These results extend the data of Iwamoto *et al.* (7) which showed that adriamycin and other quinones inhibited succinoxidase and NADH-oxidase. The potential of CoQ in cancer treatment has been reviewed by Folkers (13), and data on the relationships between CoQ_{10} and adriamycin for mitochondrial enzymes of bioenergetics have been reported (14). Kishi and Folkers (4) described data on the prevention by CoQ_{10} of the inhibition by adriamycin to CoQ_{10}-enzymes in mitochondria from beef heart tissue.

Bertazzoli *et al.* (15) reported upon the antagonistic action

of ubiquinone (CoQ_{10}) on the cardiotoxicity of adriamycin in the isolated rabbit heart system. Bertazzoli *et al.* (16) reported that the cardiotoxicity of adriamycin in rabbits was prevented by ubiquinone (CoQ_{10}). Gosalvez *et al.* (17) found that adriamycin and daunorubicin inhibited mitochondrial respiration and that state 3, but not state 4, was inhibited. Bachmann *et al.* (18) reported that adriamycin and daunomycin were the most potent of seven anthracycline antibiotics for damage of mitochondrial function, and that the most toxic compounds induced intraventricular block, bradycardia, and heart failure.

Iwamoto *et al.* (7) and Kishi and Folkers (4) showed that adriamycin was about four times as inhibitory as daunomycin to succinoxidase. The fact that adriamycinone was about three times as inhibitory as adriamycin to succinoxidase may be because adriamycinone is more lipoidal than adriamycin, and CoQ_{10} is lipoidal. The additional hydroxyl group of adriamycin may be critical for binding at the receptor for CoQ_{10}, since it may not affect the electron transfer nature of rings B and C.

The antitumor activity of adriamycin might be largely due to intercalation within DNA helices (19), and possibly to a minor inhibition of CoQ_{10}-enzymes in tumor tissue (20). The cardiotoxicity of adriamycin might be largely due to inhibition of CoQ_{10}-enzymes that are present at a high level in cardiac tissue and that function in bioenergetics.

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