# INHIBITION OF COENZYME $Q_{10}$-ENZYMES, SUCCINOXIDASE AND NADH-OXIDASE, BY ADRIAMYCIN AND OTHER QUINONES HAVING ANTITUMOR ACTIVITY*

by

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SUMMARY

Adriamycin, carminomycin, and daunorubicin inhibit the coenzyme $Q_{10}$-enzymes, succinoxidase and NADH-oxidase. Adriamycin 14-octanoate, which is more lipoidal than adriamycin, was the most effective inhibitor of the anthracyclines for both enzymes, and was 1/12 as effective as the standard inhibitor, 6-ω-cyclohexylpentyl-5-hydroxy-2,3-dimethoxy-1,4-benzoquinone, of coenzyme $Q_{10}$ for NADH-oxidase. Lapachol and dichloroallyl lawsone inhibited succinoxidase, and the latter of all quinones was second only to the standard for inhibition.

These data indicate that the antitumor activities of adriamycin could possibly be partly due to inhibition of $CoQ_{10}$-enzymes in electron transfer processes of cell respiration in addition to intercalation within DNA helices.

## INTRODUCTION

The anthracycline quinones, adriamycin and daunorubicin, are among the most promising new antitumor agents in clinical medicine at the present time, and have been clinically used for the treatment of leukemias (1,2). Adriamycin has also been extensively administered to patients having a number of solid tumors (3,4). Adriamycin also appears to be useful in the treatment of Ewing's sarcoma (5). It is believed that the antineoplastic activities of these quinones stem from their interaction with nucleic acids (6,7), and it was proposed that intercalation of the anthraquinone moiety between base pairs of the DNA helix occurs with subsequent inhibition of DNA replication and/or RNA synthesis. Apparently, the free amino group in the sugar moiety is necessary for DNA binding. Both adriamycin and daunorubicin inhibit reverse transcriptases (8) of various tumor viruses.

In these tetracyclic anthracyclines, rings B and C of adriamycin, daunorubicin, and carminomycin are depicted as quinoid and hydroquinoid, respectively, but these two rings are tautomeric. Since this structural feature is prominent, in a functional sense, in these complex antineoplastic agents, it was considered important to study them for possible inhibition of coenzyme $Q_{10}$ at its sites in mitochondrial $CoQ_{10}$-enzymes and related $CoQ_{10}$-enzymes of the Golgi apparatus.

* Coenzyme Q.180. Antimetabolites of Coenzyme Q. 25.

Brazhnikova et al. (9) described carminomycin in 1974. Consequently, adriamycin, daunorubicin, and carminomycin were initially tested and found to show some inhibition in comparison with a standard inhibitor of coenzyme $Q_{10}$. Lapachol and dichloroallyl lawsone were also assayed, because they are also quinones and have a background of information on their antitumor activities.

On finding that adriamycin is inhibitory, adriamycin 14-octanoate was then assayed, because it is considerably more lipoidal (perhaps up to 1000-fold) than adriamycin according to the concepts of Hansch (10) and Leo et al. (11). The formulas of these quinones are in Figure 1.

## MATERIALS AND METHODS

Beef heart mitochondria were prepared as described (12). The final mitochondrial pellet was suspended in 0.25 M sucrose. Phospholipid micelles were prepared by sonication of commercial soybean phospholipids (Asolectin) (13). Protein was determined by the method of Lowry et al. (14). Succinoxidase and NADH-oxidase activities were determined manometrically in a Gilson differential respirometer. The background of assay of succinoxidase and NADH-oxidase has been described (15). The reaction mixture in each flask contained 1.6 ml of 0.1 M Tris-HCl buffer, pH 7.5; 0.5 ml of 1 M sucrose; 0.05 ml of Asolectin (20 mg/ml); 0.1 ml of inhibitor dissolved in distilled water; 0.05 ml of 0.2% cytochrome c; mitochondrial enzyme (0.684 mg of protein or 0.570 mg of protein); and 0.2 ml of 0.75 M succinate or 0.07 M NADH in a total volume of 2.8 ml. The standard was dissolved in 0.1 ml of ethanol.

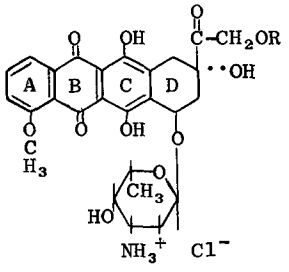
6-$\omega$-Cyclohexylpentyl-5-hydroxy-2,3-dimethoxy-1,4-benzoquinone served as a standard inhibitor, because of its strong and stable inhibitory activity in succinoxidase and NADH-oxidase in beef heart mitochondria as reported (16). To compare the inhibitory activities of the test quinones, the inhibitory activities are expressed as an antimetabolite $CoQ_{10}$ index (17). This index is calculated on the basis of the nmoles of the inhibitor per nmole of $CoQ_{10}$ for approximately 50% inhibition of enzyme activity. The amount of $CoQ_{10}$ in the mitochondria preparation was determined by the Craven's reaction (18) after extraction with pentane (18). The mitochondria contained 3.37 nmoles of $CoQ_{10}$ per mg of mitochondrial protein.

## RESULTS AND DISCUSSION

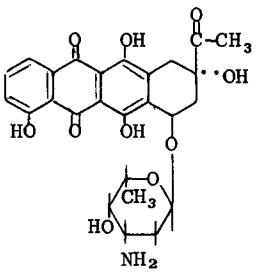
The data on the inhibition of the activities of succinoxidase and NADH-oxidase in mitochondria from beef heart are in Table 1. Alanine methyl ester hydrochloride was used as a substance for a negative control at a concentration comparable to that used for the quinones, and it is evident that the activity of the succinoxidase was not significantly affected.

Of the three anthracycline quinones, adriamycin showed greater inhibition

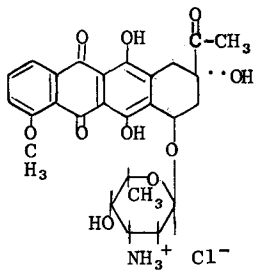
Figure 1. QUINONE RELATIONSHIPS



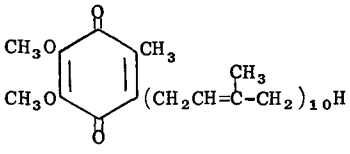
Adriamycin·HCl; R = H
Adriamycin 14-Octanoate·HCl;
R = $\overset{O}{\overset{\|}{C}}(CH_2)_6CH_3$



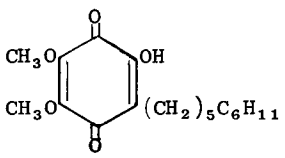
Carminomycin



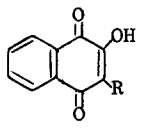
Daunorubicin·HCl



Coenzyme $Q_{10}$



Standard $CoQ_{10}$ Inhibitor
$CoQ_{10}$-Index = 5



Lapachol, R = $CH_2CH{=}\overset{CH_3}{\overset{|}{C}}{-}CH_3$
Dichloroallyl Lawsone;
R = $CH_2CH{=}\overset{Cl}{\overset{|}{C}}{-}Cl$

than carminomycin and daunorubicin, and the latter quinone showed the weakest inhibition. Their $CoQ_{10}$-indices were 1475, 2473, and 3818, respectively. The standard inhibitor was 6-$\omega$-cyclohexylpentyl-5-hydroxy-2,3-dimethoxy-2,4-benzoquinone which had a CoQ-index of 5. In comparison with this index of the standard, the ratio of the indices for adriamycin, carminomycin, and daunomycin were 295, 495, and 764, respectively. These three anthracycline quinones differ only in relatively minor structural features as shown in Figure 1, but these differences could effect the redox potential which is important for inhibition of electron transfer in $CoQ_{10}$-enzymes. Each anthracycline possesses a tautomeric quinone-hydroquinone functionality in rings B and C which can participate in oxidation-reduction reactions and possibly in $CoQ_{10}$-enzyme systems of the mitochondrial electron transfer processes.

Effective inhibitors of $CoQ_{10}$-enzyme systems appear to require a lipoidal nature (17,19), because coenzyme $Q_{10}$ is lipoidal. Consequently, adriamycin 14-octanoate was tested in the succinoxidase system and found to be about 2.5-times more inhibitory than adriamycin; its CoQ-index was 623.

The importance of aliphatic side chains for the lipoidal nature of such quinones as $CoQ_{10}$-inhibitors is substantiated by the finding that side chains of a-

Table 1. INHIBITION OF SUCCINOXIDASE ACTIVITY IN BEEF HEART MITOCHONDRIA

| Compounds | Concentrations (μ moles)[1] | Specific Activity[2] | Relative Activity (%) | Antimetabolite CoQ-Index[3] | Ratio of Indices Compound / Standard |
|---|---|---|---|---|---|
| Control | ---- | 0.349 | 100 | ---- | ---- |
| Alanine methyl ester·HCl | 5.0 | 0.340 | 97 | | |
| Standard inhibitor of $CoQ_{10}$[4] | 0.016 | 0.159 | 46 | 5 | 1 |
| Adriamycin·HCl | 1.0 | 0.343 | 98 | 1475 | 295 |
| (NSC 123127) | 5.0 | 0.063 | 18 | | |
| Adriamycin 14-octanoate·HCl | 2.5 | 0.022 | 6 | 623 | 125 |
| (NSC 149584) | 0.5 | 0.294 | 85 | | |
| Carminomycin | 1.0 | 0.332 | 95 | 2473 | 495 |
| (NSC 180024) | 6.0 | 0.167 | 48 | | |
| Daunorubicin·HCl | 1.89 | 0.331 | 95 | | |
| (NSC 82151) | 6.0 | 0.277 | 79 | 3818 | 764 |
| | 12.0 | 0.096 | 27 | | |
| Lapachol | 1.0 | 0.220 | 63 | 1035 | 207 |
| (NSC 11905) | 6.0 | 0.175 | 50 | | |
| Dichloroallyl lawsone | 1.0 | 0.176 | 50 | 296 | 59 |
| (NSC 126771) | 6.0 | 0.087 | 25 | | |
| **INHIBITION OF NADH-OXIDASE ACTIVITY IN BEEF HEART MITOCHONDRIA** | | | | | |
| Control | ---- | 0.453 | 100 | ---- | ---- |
| Standard inhibitor of $CoQ_{10}$[4] | 0.039 | 0.162 | 39 | 20 | 1 |
| Adriamycin·HCl | 2.0 | 0.118 | 26 | 772 | 39 |
| | 0.4 | 0.400 | 88 | | |
| Adriamycin 14-octanoate·HCl | 0.8 | 0.042 | 9 | 237 | 12 |
| | 0.4 | 0.218 | 48 | | |

[1] Concentration in reaction vessel which contained 0.684 mg of mitochondrial protein for succinoxidase activity determination and 0.570 mg of mitochondrial protein for NADH-oxidase activity assay.

[2] μ atoms $O_2$/min/mg protein.

[3] Antimetabolite CoQ index is defined as the ratio of nmoles of inhibitor to nmoles of $CoQ_{10}$ in the mitochondria preparation which causes approximately 50% inhibition of enzyme activity. Fifty percent inhibition is sometimes estimated by extrapolation.

[4] 6-ω-cyclohexylpentyl-5-hydroxy-2,3-dimethoxy-1,4-benzoquinone.

bout 15 carbon atoms in alkylated 6-hydroxy-5,8-quinolinequinones imparts maximal activity, in vivo, against Plasmodium berghei in mice and, in vitro, in succinoxidase and NADH-oxidase enzyme systems (20,17). For alkylmercapto side chains, maximal activity for prophylaxis against Plasmodium gallinaceum in chicks in a series of 7-alkylmercapto-6-hydroxy-5,8-quinolinequinones was found in the 7-n-heptadecylmercapto derivative (21).

Lapachol and dichloroallyl lawsone were tested only for inhibition of succinoxidase. Dichloroallyl lawsone had a CoQ-index of 296 in comparison with 1035 for lapachol, and their ratios of indices in comparison with the standard are 59 and 207, respectively. Dichloroallyl lawsone was five times more active than adriamycin in inhibition of succinoxidase, but only one-sixtieth as effective as the standard.

In the NADH-oxidase system, adriamycin 14-octanoate had a CoQ-index of 237 in comparison with 772 for adriamycin. Their ratios of indices in comparison with the standard are 12 and 39, respectively.

These data on the anthracycline quinones, particularly on adriamycin, indicate that their antitumor activities could be partly due to inhibition of one or more $CoQ_{10}$-enzyme systems of the electron transfer reactions of the mitochondrion and the Golgi apparatus in the cell in addition to intercalation within DNA helices.

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