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Rescue by coenzyme Q_{10} from electrocardiographic abnormalities caused by the toxicity of adriamycin in the rat*

(cardiotoxicity/cancer/electron transfer/ubiquinone)

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ABSTRACT **The administration of adriamycin to rates increased ($P < 0.01$) the interval, measured in msec, of the electrocardiographic QRS traces in rats, and the magnitude of the increase was *ca.* 50%. The administration of coenzyme Q_{10} to such adriamycin-treated rats allowed "rescue" or restoration of a normal QRS complex after 7 days of administration of coenzyme Q_{10}. The QRS complex then remained normalized during the subsequent period of 21–30 days, by which time the cumulative dose of adriamycin had reached 24 mg/kg. Also, the QRS interval was lower ($P < 0.01$) on day 33 than it was for rats treated to the same day with adriamycin alone. Coenzyme Q_{10} offers promise of rescue from at least some of the cardiotoxicity occurring in adriamycin-treated cancer patients, probably by a similar mechanism to that of the clinical rescue from toxicity of methotrexate by a cofactor of folic acid (citrovorum factor).**

The cardiotoxicity of adriamycin to cancer patients has become a widely recognized side effect and has resulted in a recommended maximum dosage of 550 mg/m^2 body surface. This cardiotoxicity has also been observed at dosage levels lower than 550 mg/m^2, and a consideration to exceed this maximum dosage has necessitated decisions on comparing the risk of tumor growth with the risk of congestive heart failure that results from the side effect. The clinical importance of adriamycin for cancer therapy has led to ongoing and diversified broad investigations toward the elucidation of the mechanism of the cardiotoxicity and its possible control.

Noting the structural relationship of adriamycin and coenzyme Q_{10} (CoQ_{10}) (both are quinones), Iwamoto *et al.* (1) found that adriamycin inhibits the CoQ_{10} enzymes succinoxidase and NADH oxidase, and Folkers (2) gave an account of the potential of CoQ_{10} in cancer treatment. Kishi and Folkers (3) demonstrated a prevention by CoQ_{10} of the toxicity of adriamycin to mitochondrial CoQ_{10} enzymes of the myocardium, and Kishi *et al.* (4) greatly extended these observations to several forms of CoQ under diversified *in vitro* conditions.

Using experimental animal systems, Bertazzoli *et al.* (5, 6) described model rabbit systems to study the cardiotoxicity of adriamycin, and then the prevention of the cardiotoxicity in this model by CoQ_{10}. Jaenke (7) reported extensively on cardiomyopathy in rabbits that was induced by adriamycin and related anthracyclines. Mettler *et al.* (8) summarized the adriamycin-induced cardiomyopathy and congestive heart failure in rats, and included histological data on myocardial changes and ultrastructural alterations. Lenaz and Page (9) reviewed the pathogenesis of the cardiomyopathy due to adriamycin and the clinical status of the cardiotoxicity.

Combs *et al.* (10) found that treatment of mice with CoQ_{10} before adriamycin administration allowed an increase ($P < 0.05$) in survival from 36% to 80% and from 42% to 86%, respectively, in two protocols. The sequential data of Zbinden *et al.* (11, 12) indicated protection by CoQ against the electrocardiographic changes in rats subjected to the cardiotoxicity of adriamycin, but these workers considered their findings only preliminary. Choe *et al.* (13) definitively demonstrated that the widening of the electrocardiographic QRS interval did not appear when CoQ_{10} had been administered to adriamycin-treated rats.

The clinical practice of "rescue" by the citrovorum factor (a coenzyme) from the toxicity of deliberately chosen high dosages of methotrexate (an enzyme inhibitor) for cancer patients is widely known. It was considered important to test for the rescue by CoQ_{10} from at least some of the cardiotoxicity of adriamycin in rats, as judged by electrocardiographic parameters. The conduct and results of these rescue experiments are described herein.

MATERIALS AND METHODS

Male Sprague–Dawley rats from the Tempco Breeding Laboratories, Houston, TX, were fed Wayne Lab Blox and water ad lib. The rats were maintained on 12-hour light–dark cycles. They weighed approximately 180–200 g when the protocol was started.

A Physiograph desk model 4A with solid-state electronics from Narco Biosystems, Houston, TX, was used to obtain the electrocardiograph recordings. Needle electrodes were inserted under the skin without anesthesia for the limb lead at position 2. The speed of the paper was 10 cm/sec. Averages of all measurements were calculated on the basis of at least five consecutive cycles. The normalcy of the electrocardiogram pattern was confirmed for each rat before the initiation of drug treatment. The electrocardiograms were recorded once or twice a week in the mornings before any administration.

Adriamycin from the Adria Laboratories, Columbus, OH, was dissolved in deionized water. The CoQ_{10} was formulated in a 10% solution of Tween-20. Intraperitoneal injections were made daily on Mondays through Fridays. Doses of adriamycin at 1 mg/2 ml per kg and CoQ_{10} at 1 mg/ml per kg were administered. The formulation of CoQ_{10} and the corresponding vehicle were administered in the mornings and the formulation of adriamycin and its corresponding vehicle were administered in the afternoons. There were three groups of rats. One group received injections of only water, the adriamycin vehicle. Another group was treated with a 10% solution of Tween-20, the vehicle for CoQ_{10}. Adriamycin was administered to the remaining group. Only those rats that developed a widening of the QRS complex at a dosage of 12 mg of adriamycin per kg were selected for the final protocol, and these rats constituted about 80% of the animals in the initial adriamycin-treated group. The remaining 20% of the animals were not used.

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Abbreviation: CoQ, coenzyme Q.

* This is paper 245 in a series on coenzyme Q.

Table 1. Control data from vehicle treatment

Water (4/21 rats)		Tween-20 (4/21 rats)	
Day	QRS, msec	Day	QRS, msec
0	15.8 ± 1.4	0	17.0 ± 1.2
14	17.0 ± 0.7	14	16.3 ± 1.4
21	16.5 ± 0.9	21	17.0 ± 1.2
28	15.0 ± 0.4	28	16.3 ± 0.8
30	15.8 ± 0.8	30	17.0 ± 0.8
33	15.3 ± 0.3	33	18.5 ± 0.5
Means	15.9 ± 0.3		17.0 ± 0.4

Injections were given 5 days per week. Results are given ±SEM.

Those rats receiving adriamycin and showing a widening of the QRS complex were divided into two groups, so that there were no significant differences between the mean values of the two groups in terms of the QRS complex or the body weight. One of these groups was treated with both CoQ_{10} and adriamycin, and the second group was continued on the treatment with adriamycin alone.

For a statistical analysis, differences of mean values were determined by the analysis of variances followed by Scheffe's method of multiple contrast (14).

RESULTS AND DISCUSSION

This study was conducted in five protocols, and the data that demonstrate the rescue by CoQ_{10} from the toxicity of adriamycin are summarized in Tables 1 through 4 as the toxicity was monitored by the QRS complex.

The 21 selected rats before any treatment had a mean value of the QRS complex of 15.5 ± 0.5 msec.

The data in Table 1 show that 4 of these 21 rats, which were then treated with water as the vehicle for adriamycin for a period of 33 days, during which time the QRS complex was monitored six times, gave a mean value of 15.9 ± 0.3 msec. Similarly, 4 other rats from the initial group of 21 were treated with Tween-20 over a period of 33 days, and, after similar monitoring of the QRS complex, a mean value of 17.0 ± 0.4 msec was obtained.

There is no significant difference between the mean values of the QRS complex for the initial group and for the two subsequent subgroups that were treated with the two vehicles.

The data in Table 2 for the remaining 13 of the 21 rats, which were treated with adriamycin alone at a dosage level of 1 mg/kg per day for 5 days a week for a total of 12 injections, or 12 mg/kg as a cumulative dose. On day 0 of this protocol, before the first dose of adriamycin, the mean value for the QRS complex was 14.9 ± 0.6 msec. On day 14, after a cumulative dose of 12 mg, the mean value of the QRS was 22.2 ± 0.7 msec, which is significantly higher ($P < 0.01$). In this manner, the toxicity of adriamycin could be monitored by the QRS complex as a criterion, and the toxicity was clearly evident.

In our previous study by Choe *et al.* (13), CoQ_{10} prevented the widening of the QRS complex and the elongation of the Q–T interval in adriamycin-treated rats.

Table 2. Treatment period with adriamycin alone

Cumulative dose, mg	Day	QRS, msec
0	0	14.9 ± 0.6
12	14	22.2 ± 0.7

Thirteen of 21 rats received 1 mg of adriamycin per kg per day, 5 days per week, for a total of 12 injections. For 14.9 vs. 22.2 msec, $P < 0.01$.

Table 3. Continued treatment with adriamycin alone

Cumulative dose, mg	Day	QRS, msec
12	14	22.0 ± 1.0
17	21	22.4 ± 1.1
22	28	21.8 ± 0.9
24	30	21.4 ± 1.0
27	33	24.0 ± 1.2
	Mean	22.3 ± 0.5

Six of the 13 rats described in Table 2 were treated as shown.

The 13 rats of Table 2 were then divided into two groups of 6 and 7 rats each, and they were treated according to the data in Tables 3 and 4.

In Table 3, the data are shown for the group of 6 rats continued on the treatment of adriamycin alone during days 14–33, during which period the cumulative dose of adriamycin increased from 12 to 27 mg/kg. The QRS complex remained as an unchanged and widened interval with a mean value of 22.3 ± 0.5 msec.

The data in Table 4 are on the group of 7 rats that were treated both with adriamycin and CoQ_{10}. These data show the rescue or the restoration of a normal QRS interval after 7 days because the value of 17.6 ± 0.5 is not statistically different from the values for rats before treatment and the two vehicle-treated groups shown in Table 1. The rescue is significant by $P < 0.01$.

It is also notable that the QRS interval remained "normalized" during the period of 21–30 days, because the mean value of 16.8 ± 0.5 msec is not different from the three control values.

It is also notable that the QRS interval on day 33, at which time the cumulative dosages of adriamycin and CoQ_{10} had reached 27 and 15, respectively, of 19.3 ± 0.7 is significantly lower ($P < 0.01$) than the value of 24.0 ± 1.2 on day 33 for the 6 rats treated with adriamycin alone.

An analogy can be made between the chemotherapy of cancer with methotrexate and rescue with the citrovorum factor, and the chemotherapy of cancer with adriamycin and rescue with CoQ_{10}.

Frei *et al.* (15) provided an extensive summary of new approaches to cancer chemotherapy with methotrexate. One of their hypotheses was that a very high concentration of methotrexate could destroy cells by a mechanism that was different from that when conventional doses were used, at least for some tumors, in a preferential destruction of tumor cells, and that the citrovorum factor may selectively rescue normal cells. Their

Table 4. Treatment period with adriamycin and CoQ_{10}

Cumulative dose, mg		Day	QRS, msec		
Adriamycin	CoQ_{10}				
12	0	14	22.4 ± 0.9		
17	5	21	17.6 ± 0.5		
22	10	28	15.5 ± 0.7	Mean,	16.8 ± 0.5
24	12	30	17.2 ± 1.1		
27	15	33	19.3 ± 0.7		

Seven of the 13 rats described in Table 2 were given 1 mg of adriamycin and 1 mg of CoQ_{10} per kg per day, 5 days per week. The mean QRS for days 21–30 is not significantly different from the 14.9 ± 0.6 msec for day 0.

experimental studies and clinical findings substantiate their hypothesis. If methotrexate may be conceptually related to adriamycin as an inhibitor, and if the citrovorum factor (coenzyme form of folic acid) may be conceptually related to CoQ_{10} as a cofactor, then we may interpret and project our data herein as follows:

Adriamycin is a quinone that is known to inhibit mitochondrial CoQ_{10} enzymes, but the antitumor activity of adriamycin is presumed to be based upon intercalation with DNA and RNA. Bertazzoli and Ghione (16) have reported that CoQ_{10} in certain animal models does not interfere with the antitumor activity of adriamycin. To the extent that the cardiotoxicity of adriamycin is due to the inhibition of CoQ_{10} enzymes, there is the prospect of protection as well as rescue of the myocardium from the toxicity of adriamycin by prophylactic or curative (rescue) treatment with CoQ_{10}. Because Combs *et al.* (10) found that an adriamycin dosage of 15 mg/kg, when administered to mice, allowed only 42% survival by day 30 after the administration of adriamycin, and that a 4-day pretreatment with CoQ_{10} before the administration of adriamycin allowed an 80% survival in mice, it is probable that the widening of the QRS complex described herein at accumulated adriamycin dose of 12–27 mg/kg is indeed a criterion of toxicity.

CoQ_{10} allowed rescue of the toxicity of adriamycin as judged by the normalization of the abnormal adriamycin-treated rats.

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