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Structural and Functional Impairment of Mitochondria in Adriamycin-Induced Cardiomyopathy in Mice: Suppression of Cytochrome *c* Oxidase II Gene Expression

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ABSTRACT. The use of adriamycin (ADR) in cancer chemotherapy has been limited due to its cumulative cardiovascular toxicity. Earlier observations that ADR interacts with mitochondrial cytochrome *c* oxidase (COX) and suppresses its enzyme activity led us to investigate ADR's action on the cardiovascular functions and heart mitochondrial morphology in Balb-c mice i.p. treated with ADR for several weeks. At various times during treatment, the animals were assessed for cardiovascular functions by electrocardiography and for heart tissue damage by electron microscopy. In parallel, total RNA was extracted from samples of dissected heart and analyzed by Northern blot hybridization to determine the steady-state level of three RNA transcripts encoded by the COXII, COXIII, and COXIV genes. Similarly, samples obtained from the liver of the same animals were analyzed for comparative studies. Our results indicated that 1) treatment of mice with ADR caused cardiovascular arrhythmias characterized by bradycardia, extension of ventricular depolarization time (tQRS), and failure of QRS at high concentrations (10–14 mg/kg body weight cumulative dose); 2) the heart mitochondria underwent swelling, fusion, dissolution, and/or disruption of mitochondrial cristae after several weeks of treatment. Such abnormalities were not observed in the mitochondria of liver tissue; and 3) among the three genes of COX enzyme examined, only COXII gene expression was suppressed by ADR treatment, mainly after 8 weeks in both heart and liver. Knowing that heart mitochondria represent almost 40% of heart muscle by weight, we conclude that the deteriorating effects of ADR on cardiovascular function involve mitochondrial structural and functional impairment. BIOCHEM PHARMACOL **57**;5:481–489, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. adriamycin; cytochrome c oxidase; cardiomyopathy

Anthracyclines are potent antitumor agents with wide clinical applications. However, their long-term clinical use is limited due to severe bone marrow suppression and cumulative dose-dependent cardiovascular toxicity. Although numerous studies have been already carried out to elucidate the pathophysiological basis of cardiotoxicity, it is still not clear which one of the proposed mechanism(s) of ADR's¶ action is the most responsible [1, 2]. Briefly, ADR-induced cardiovascular toxicity was attributed on the one hand to free radical formation and membrane lipid peroxidation [3–5], and on the other to gradual impairment of mitochondrial function. Indeed, several studies have confirmed the involvement of mitochondria in the pathogenesis of ADR-induced cardiotoxicity [1, 6–11], even though it has never been fully elucidated how this cytopathic effect really occurs at the level of these organelles. It has been shown that administration of ADR reduces cell respiration, inhibits oxidative phosphorylation, decreases mitochondrial ATPase activity, and affects the redox state of respiratory carriers [12, 13]. These functional alterations are reflected in the ultrastructural appearance of mitochondria, possibly due to a general disturbance of the inner mitochondrial membrane structure and function [14] and a decrease in membrane fluidity [15].

Most recently, we observed that daunomycin and ADR interact specifically with mitochondrial cytochrome *c* oxidase (COX), a pivotal mitochondrial enzyme for cell respiration, and suppress its activity [16]. Based on these findings, we considered COX as a novel target enzyme involved in ADR-induced cardiovascular toxicity. Furthermore, we demonstrated that ADR selectively prevents the expression of COXII gene of mitochondrial

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¶ *Abbreviations:* ADR, adriamycin; COX, cytochrome *c* oxidase; bw, body weight; ECG, electrocardiogram; tQRS, duration of QRS complex; and R-R interval, time between peaks of ventricular depolarization.

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origin and promotes apoptosis [17] in human leukemia K-562 cells.

In this study, we first evaluated the cardiovascular effects of ADR in Balb-c mice by electrocardiography and electron microscopy and then correlated them with possible effects of ADR on the expression of COXII, COXIII, and COXIV genes encoding three subunits of COX enzyme. This animal study could enable us to delineate the mechanism(s) of ADR-induced cardiomyopathy seen in cancer patients undergoing ADR treatment.

MATERIALS AND METHODS

Chemicals and Biologicals

Adriblastina (ADR.HCl) purchased in the form of lyophilized powder (containing 10 mg of ADR.HCl, 50 mg of lactose, and 1 mg of methylparaben) from Farmitalia Carlo Erba was reconstituted prior to injection. DEPC (diethyl pyrocarbonate), guanidine, thiocyanate salt and sarcosyl (*N*-lauroyl-sarcosine) were purchased from SIGMA, sodium citrate from Mallinckrodt, and 2-mercaptoethanol from BDH Chemicals Ltd. SDS was purchased from SERVA. Ultra pure agarose was purchased from BRL, Life Technologies Inc. Hybridization transfer membranes HYBOND-N, the Multiprime DNA Labeling System, and radioisotope [a-^{32}P]-dCTP were purchased from Amersham and isoflurane (FORENIUM™) from Abbott. All chemicals used for electron microscopy study were purchased from Agar Scientific.

Animals

Female Balb-c mice, 18–24 g, used throughout this study were bought from the Theageniom Anticancer Institute of Thessaloniki and bred in our animal house.

DNA Probes

A full-length human liver cDNA clone, pCOX4.111 (700 bp), corresponding to subunit COX IV (**pCOX41**) [18] and the oligonucleotide primers Pst9020-F (CCGCTAACAT TACTGCAGGCCACCTACTC) and Xba10256-B (GGG CAATTTCTAGATCAAATAATAAGAAGG) used for polymerase chain reaction amplification of the mitochondrial COXIII (plus part of ATPase 6, ND3 as well as tRNA^{Gly}) gene (probe **COXIII**, 942bp) were kindly donated by Dr. Eric Schon (Columbia University, Dept. of Neurology). Another cDNA probe (451 bp) from rat pituitary tumor cells, containing a piece of COXII (**pCOXII**), was kindly donated by Dr. Priscilla Dannies (Yale University, Dept. of Pharmacology) [19]. Finally, a cDNA of 426 bp coding for the rat GAPDH [20] was kindly provided by Dr. Pantopoulos (EMBL, Germany).

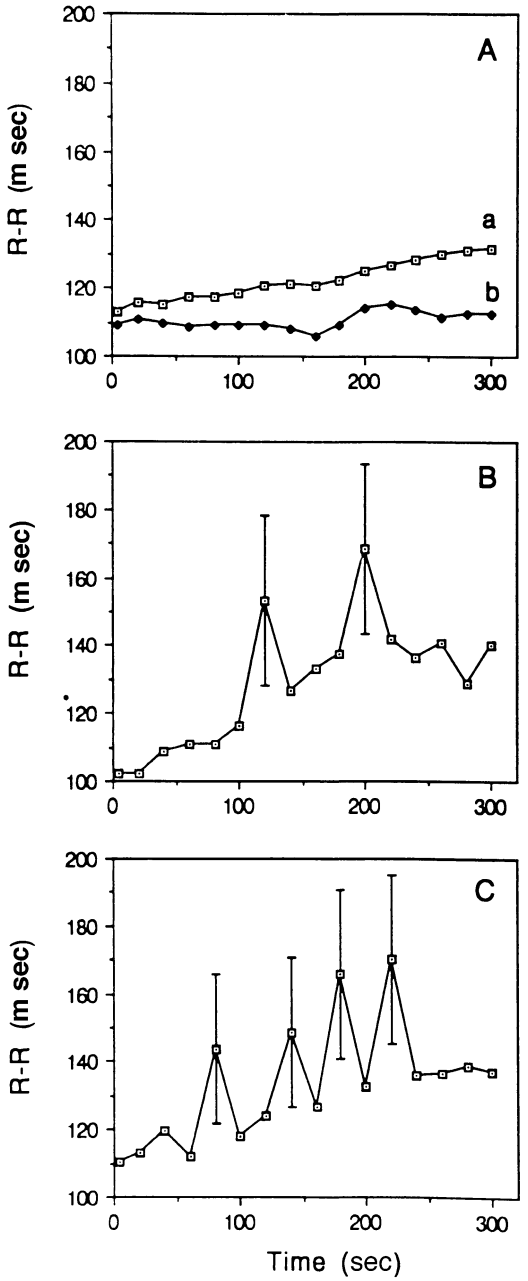

FIG. 1. Changes in R-R interval in control and ADR-treated animals. (A) The R-R interval (msec) versus time plots for two control mice with (a) or without (b) bradycardia. (B, C) The R-R interval (msec) versus time plots for two mice treated with ADR for 6 weeks. Each point represents the average value of the first four consecutive cardiac cycles, obtained every 20 sec. Standard error bars were not placed on each time point since most were below ±1%. However, bars were placed on those points which exhibited QRS failure.

Experimental Protocol

Mice were randomly divided into two main groups. One group (control animals) received 0.9% NaCl solution i.p. once a week. The other group was injected once per week, i.p., with 1 mg/kg of ADR for 6, 8, 9, 10, 12, and 14 weeks, according to Alderton *et al.* [21]. These experimental conditions were similar to those observed in patients

TABLE 1. Effect of ADR treatment on QRS failure in mice treated with ADR

Cumulative dose of ADR (mg/kg bw)	Number of mice examined	QRS failure		
		(−)	(+)	(+++)
0 (None)	(13)	13/13	0/13	0/13
6	(11)	5/11	4/11	2/11
8	(6)	1/6	5/6	0/6
9	(6)	2/6	2/6	2/6
10*	(6)	4/5	1/5	0/5
12	(6)	2/6	1/6	3/6
14*	(6)	2/5	1/5	2/5

*Five out of six animals were examined, since one of them died during the recording period.

undergoing treatment with ADR for several months. At various weekly intervals and 6 days following the last injection, mice were examined by ECG recordings (see below) and then starved for up to the following day. Then, animals were killed by cervical dislocation and hearts and livers were removed immediately. These tissues were dissected right away (~1 min) and slices were prepared for electron microscopy. Total RNA was extracted from the rest of the tissues immediately. Samples of livers were also examined for comparative studies, since liver damage has not been reported under ADR treatment. The total cumulative dose of ADR was expressed in mg of ADR per kg body weight (mg/kg bw). No substantial differences in body weight were observed between control (untreated) and ADR-treated animals.

Electrocardiographic (ECG) Evaluation of Animals

To assess the effect of ADR on cardiovascular functions, mice were anesthetized first with ethyl ether (30–40 sec) and then maintained in deep anesthesia using isoflurane. For each mouse examined, ECG recording (lead II) started 2–3 min following deep anesthesia and lasted for about 300 sec. ECG recordings (1 sec in duration) were stored in a PC every 4 sec for further analysis.

Cardiovascular toxicity induced by ADR was judged on the basis of changes seen: a) in the R-R interval (time elapsed from the peak of one ventricular depolarization to the next one). Within the 300-sec period of ECG recording, the R-R interval was assessed from the first four consecutive cardiac cycles, obtained every 20 sec. The values were measured (in msec), averaged, and a plot of mean values versus time was constructed for each animal (Fig. 1); and b) in the duration of QRS complex (tQRS), corresponding to the time of ventricular depolarization. The latter is one of the most useful parameters to identify ADR-induced cardiotoxicity [22–24]. The tQRS complex (msec) was measured and averaged from the first 10 successive cardiac cycles at the beginning (t = 1 sec) as well as at the end of the recording period (t = 300 sec). Both parameters (R-R interval and tQRS) were expressed as mean values ± SD and analyzed by student *t*-test ($P < 0.05$). Anesthesia had no effect on the health of mice examined, since all control animals recovered well after 10–15 min following anesthesia. In contrast, a number of animals treated with ADR for 10–14 weeks died during or after anesthesia, presumably because anesthesia potentiated problems already induced by ADR treatment.

Electron Microscopy

Samples of the heart (left ventricular wall) and liver tissues were cut into 1 mm^3 pieces and placed in cold Carnovsky's fixative [25], pH 7.4, at 4° for 1 hr. After washing in 0.2 M phosphate buffer, pH 7.4, the tissues were postfixed in 2% OsO_4, pH 7.2, at 4° for 30–60 min, washed in distilled water, and stained *en bloc* in a 0.5% aqueous uranyl acetate solution, pH 4.5, for 16 hr at 4°. Following dehydration in a graded ethanol series, the tissues were infiltrated and embedded in Spurr's resin [26]. Polymerization was performed at 70° for 9 hr. Silver to pale gold sections were prepared using a Reichert OM-3 ultramicrotome, stained with lead citrate [27], examined and photographed in an IEOL 100B electron microscope operating at 80 kV.

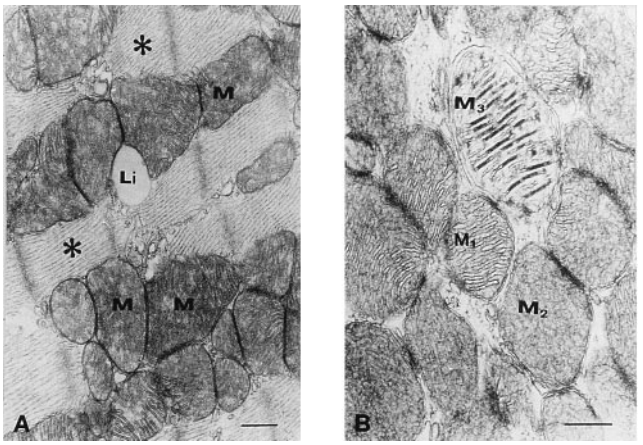

FIG. 2. Ultrastructure of cardiac cells in ADR-treated animals. Samples of heart tissue, removed from animals treated with ADR for 12 weeks, were analyzed by electron microscopy. (a) Several mitochondria (M) exhibit swelling, fusion and/or disruption of cristae, while the myofibrilar organization (asterisks) remains unchanged (×30,000; Li = lipid). (b) Cardiac mitochondria display altered morphology. M1 has normal cristae, M2 has no cristae at all, while M3 has electron-dense cristae and an electron-lucent matrix (×40,000). Bar represents 0.3 μm.

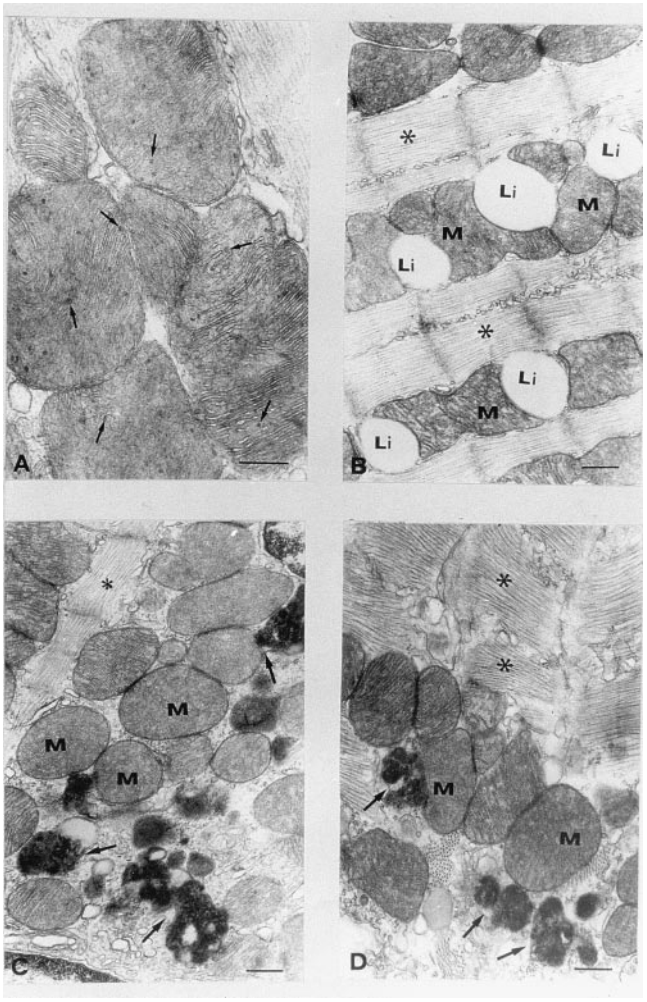

FIG. 3. Ultrastructure of cardiac cells in ADR-treated animals. (A) Numerous intramitochondrial granules (arrows) are evident after 14 weeks of ADR treatment (14 mg/kg bw) (×40,000) (B) After 8 weeks of ADR treatment, a large number of lipid droplets (Li) are seen in close contact with mitochondria (M). Myofibrils (asterisks) display normal arrangement (×30,000). (C) After 14 weeks of ADR treatment (14 mg/kg bw), numerous lysosomes (arrows) are seen. The mitochondrial cristae are not evident in many mitochondria (M). The organization of myofibrils (asterisks) appears normal (×30,000). (D) In one animal, after 8 weeks of ADR treatment, many lysosomal profiles (arrows) are evident and many mitochondria (M) display alterations in their morphology. The organization of myofibrils (asterisks) appears normal (×30,000). Bar represents 0.3 μm.

RNA Isolation and Northern Blot Hybridization Analysis

Total RNA was extracted and purified according to the acid guanidium thiocyanate-phenol-chloroform extraction method of Chomczynski and Sacchi [28]. Briefly, portions of the heart and/or liver tissue were homogenized in 7 mL of denaturing solution with a glass-Teflon homogenizer (Heidolph RZR O) for ~1 min. The homogenates were extracted first with acid phenol for 15 min at 0° and then with chloroform: isoamyl alcohol (49:1) by vigorous shaking for ~10 sec. Formaldehyde-denatured RNA samples (10 or 15 μg of each heart or liver RNA, respectively) were electrophoretically separated on 1.2% agarose-2.2 M formaldehyde gel (~3V/cm for 12 hr), transferred onto a nylon membrane in 20x SSC (3 M sodium chloride, 0.3 M of sodium citrate, pH 7.0) for 48 hr [29] and immobilized by UV light for 5 min. Hybridization was performed according to the method of Church and Gilbert [30]. The filters were washed and autoradiographed using Kodak XAR-5 film. The films were exposed at low temperature (−70°) and autoradiographs were obtained. Nylon membranes were stripped of the hybridized probe by immersion in 50 mM of PO_4/1% SDS (pH 7.2) at 95° in order to be re-hybridized.

RESULTS

Induction of Cardiovascular Arrhythmias by Treatment of Balb-c Mice with ADR for Several Weeks

CHANGES IN R-R INTERVAL AND QRS FAILURE. Analysis of the ECG during the 300 sec recording period showed a progressive increase in the R-R interval, indicating development of bradycardia (Fig. 1A). By the end of the recording period (t = 300 sec), the R-R interval reached values which varied from 10% (Fig. 1Ab) to 40% (Fig. 1Aa), as compared to values seen at the start of the recording period (t = 1 sec). In animals treated with ADR, this increase in the R-R interval was much higher compared to that of controls. An increase in the R-R interval over the control values was observed in 3 out of 6 and 3 out of 5 mice treated with ADR for 12 and 14 weeks (12 and 14 mg/kg bw), respectively. Interestingly, a high rate of mortality (2 out of 6 and 5 out of 6) was observed in mice treated with ADR for 10 and 14 weeks. Therefore, both bradycardia and a high rate of mortality (considered to be severe symptoms of cardiotoxicity) were observed mainly in mice treated with high cumulative dose of ADR (>10 mg/kg bw). In addition to the alterations seen in the R-R interval, QRS failure (loss) was observed in a number of mice during the 300-sec recording period. When a QRS failure appeared in the ECG records, the value of the R-R interval increased sharply (almost doubled), thus yielding a higher averaged value, derived from four successive cardiac cycles (Fig. 1B, C). In contrast to treated animals (Fig. 1B, Table 1), control mice never exhibited a QRS failure [Fig. 1A, marked also as (−) in Table 1]. QRS failure was also apparent in half of the mice (6/11) treated with 6 mg of ADR/kg bw. As the cumulative dose of ADR increased, a higher number of mice with QRS failure was observed. Overall, the proportion of mice with QRS failure increased at high concentrations of ADR (12–14 mg/kg bw).

PROLONGATION OF QRS COMPLEX (tQRS). For each animal examined, the tQRS complex was always measured at the start (t = 1 sec) as well as at the end of the recording period (t = 300 sec). No significant difference between the control and ADR-treated mice ($P = 0.561$) was observed at the start of the recording period, as was mentioned above. However, a comparison of the tQRS values mea-

TABLE 2. Adriamycin-induced morphological changes in heart tissue of Balb-c mice

Cumulative dose of ADR (mg/kg bw)	Number of mice examined	Ultrastructure morphology
0.9% NaCl (control)	(5)	Normal
6	(2)	Numerous mitochondrial cristae; Few lipid droplets.
8	(3)	Numerous cristae and medium electron density of mitochondrial matrix. Sporadic fusion of the mitochondrial cristae. More lipid droplets and appearance of small number of lysosomes
9	(2)	Numerous cristae and medium electron density of mitochondrial matrix. Sporadic fusion of the mitochondrial cristae. Numerous lipid droplets.
10	(2)	Marked fusion of mitochondrial cristae.
12	(4)	Pronounced fusion of mitochondrial cristae. More abnormal mitochondrial profiles.
14	(3)	Pronounced fusion of mitochondrial cristae. Numerous intramitochondrial granules and abundant lysosomes.

sured at the start of the recording period (t = 1 sec) with those recorded at the end of this period (t = 300 sec) showed a progressive increase. This increase in tQRS was relatively small (<10%) in the control animals and higher (10–30%) in most ADR-treated mice. Prolongation of the tQRS by 10–20% over the control values was observed in almost half (5/11) of the mice treated with 6 mg of ADR/kg bw. This prolongation of the tQRS was more intense (20–30%) in half of the animals treated with ADR for longer periods (12 and 14 weeks) (data not shown).

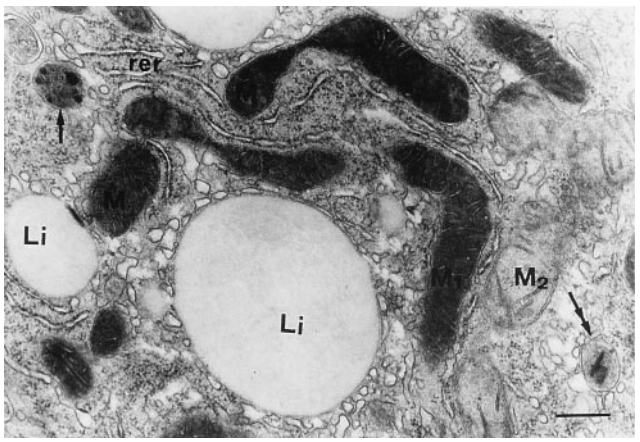

FIG. 4. Ultrastructure of hepatocytes in animals treated with ADR for 12 weeks. The morphology of mitochondria (M) in hepatocytes was normal; however, in the same samples there were neighboring mitochondria, such as M1 and M2, with different electron density of their matrices and a great number of lipid droplets (Li). The arrow points to a lysosome and the double arrow to a peroxisome (×30,000; rer = rough endoplasmic reticulum). Bar represents 0.3 μm.

Morphological Abnormalities in Mitochondria of Heart and Liver of ADR-treated Animals

Heart and liver samples removed from the same ADR-treated animals were examined by electron microscopy. Only heart mitochondria exhibited intensive morphological changes after 10 weeks of ADR treatment (10 mg/kg bw). These changes included mitochondrial swelling, fusion, dissolution and/or disruption of mitochondrial cristae (Fig. 2A). In some cases, the mitochondrial cristae were electron-dense, while the mitochondrial matrix was electron-lucent (Fig. 2B). By the 8th to 9th week of ADR treatment, many lipid droplets (Fig. 3B) were seen in the myocardial cells, while by the 12th week intramitochondrial granules (Fig. 3A) and the number of lysosomes (Fig. 3C) had been considerably increased. No substantial changes were observed in the myofibrillar network or the chromatin, whereas the number of the specific granules in the atrial myocytes remained constant (Table 2). We did not observe significant morphological changes in the liver of the ADR-treated animals. Occasionally, neighboring mitochondria displayed different electron density in their matrix. A notable increase in the number of cytoplasmic lipid droplets after 12 mg of ADR/kg bw was detected (Fig. 4).

ADR-Suppressed Expression of the COXII Gene in Mitochondria

To correlate the abnormalities seen in heart electrophysiological function and mitochondrial morphology in ADR-treated animals with possible impairment of COX enzyme, we performed Northern blot hybridization studies with four probes corresponding to two mitochondrial COX genes (COXII and COXIII), one nuclear COX gene (COXIV), and one housekeeping gene GAPDH. The data were

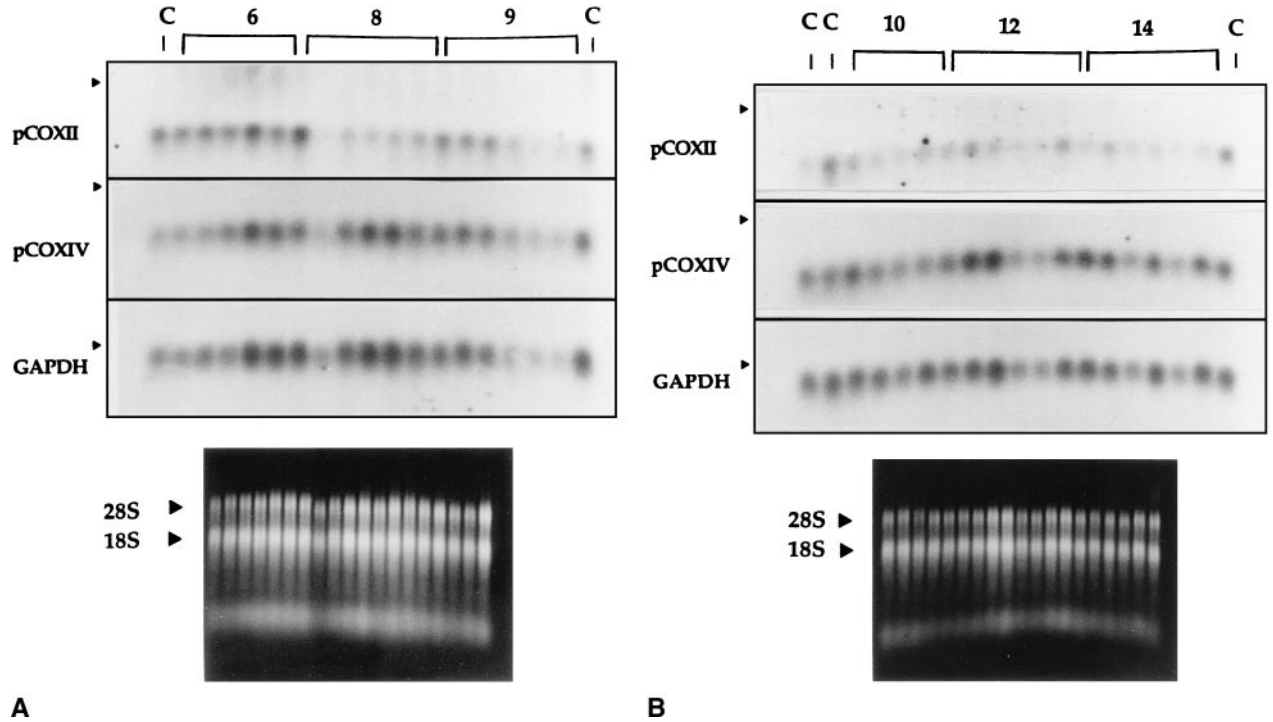

FIG. 5. ADR-induced cumulative dose-dependent effects on COXII and COXIV gene expression in heart tissue. Ten μg of RNA samples isolated from heart tissue of control or ADR-treated mice was electrophoretically separated on a 1.2% agarose-2.2 M formaldehyde gel, transferred onto a nylon membrane, and hybridized separately, first at 65° with [^{32}P]-labeled mitochondrial COXII DNA probe (pCOXII), then at 64° with [^{32}P]-labeled DNA fragment (pCOX41) coding for nuclear COXIV mRNA, and finally at 65° with [^{32}P]-labeled DNA fragment coding for GAPDH mRNA. Each time the membrane was washed and autoradiographed. The RNA samples isolated from individual animals treated either with saline (c) or ADR for a different number of weeks (6–14) are numbered at the top of figure in the upper panel. Arrowheads indicate the position of 18 S rRNA. The lower panel shows the ethidium bromide staining pattern of electrophoresed RNA and the positions of 28S and 18S rRNAs. The left panel indicates data from animals treated for 6–9 weeks and the right panel samples obtained from animals treated for 10–14 weeks.

normalized by densitometric scanning analysis (ratio of the levels of COXII or COXIV mRNA transcripts to that of GAPDH). ADR treatment led to reduction in the steady-state level of COXII RNA transcripts after 8 weeks of treatment (8 mg/kg bw). This decrease in mitochondrial COXII gene expression was rather specific, since neither the nuclear COXIV gene nor the mitochondrial COXIII gene were affected by ADR treatment (Figs. 5, 6). Similar studies performed with RNA samples removed from the liver tissues of the same animals indicated the same pattern of expression of the genes examined (Fig. 7). This suggests that mitochondrial COXII gene expression is selectively suppressed by ADR after 8 weeks of treatment in both heart and liver.

DISCUSSION

ADR-induced cardiomyopathy has long been a serious drawback in treating human cancers effectively. Despite the plethora of information accumulated thus far, the precise mechanism(s) underlying the cardiovascular effects of ADR are not known. Although the theory of free radical formation has been considered as a major mechanism for the destructive process initiated by ADR on the heart, there are still contradictory results concerning the ability of free radical scavengers to prevent ADR-induced cumulative cardiotoxicity [31–33]. This suggests that ADR may act via more than one mechanism to promote cardiovascular toxicity. Our recent observation that mitochondrial COX, a pivotal enzyme in cell respiration and energy production, serves as a novel target site for ADR's action [16] prompted us to re-examine the effects of ADR and correlate them with the expression of COX genes in an animal study. We exposed animals to ADR once per week (1 mg/kg bw) and investigated whether chronic treatment with ADR affects: (a) heart function, (b) morphology and structural integrity of heart cells, and (c) expression of three genes coding for the three subunits of COX enzyme (COXII, COXIII, and COXIV).

Electrocardiographic evaluation of ADR-treated animals indicated prolongation of the R-R interval (indicating bradycardia) as well as QRS failure and extension of tQRS after treatment with ADR for several weeks. These data are in agreement with previous studies [23] where a 30% prolongation of the tQRS was also observed in rats treated with ADR (5 mg/kg bw]. In mice treated with higher doses of ADR (12–14 mg/kg bw), the symptoms of cardiotoxicity became more intensive, since prolongation of tQRS and

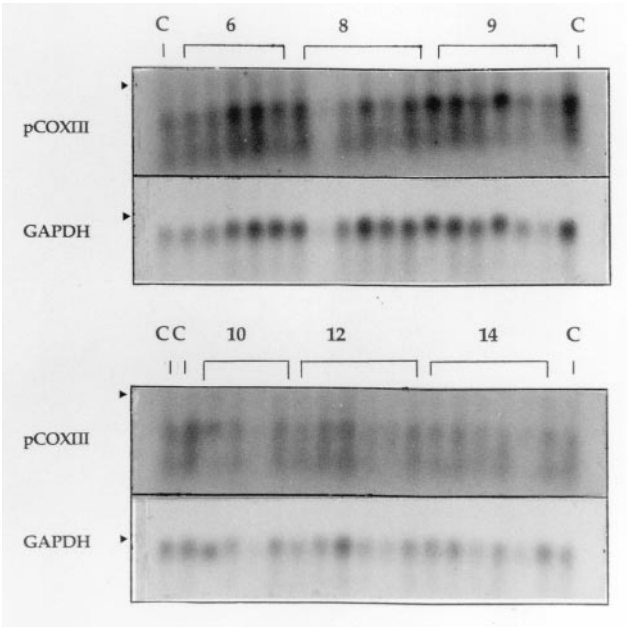

FIG. 6. ADR-induced cumulative dose-dependent effects on COXIII gene expression in heart tissue. Ten μg of RNA samples isolated from heart tissue of control or ADR-treated mice was electrophoretically separated on a 1.2% agarose–2.2 M formaldehyde gel, transferred onto a nylon membrane, and hybridized first at 63° with [^{32}P]-labeled PCR probe complementary to COXIII mitochondrial DNA, as discussed under Methods, and then at 65° with [^{32}P]-labeled DNA fragment coding for GAPDH mRNA. Each time the membrane was washed and autoradiographed. The RNA samples isolated from individual animals treated either with saline (c) or ADR for a different number of weeks (6–14) are numbered at the top of the upper and lower panel. Arrowheads indicate the position of 18S rRNA.

QRS failure had been more frequent. Furthermore, there was a high rate of mortality in animals treated with ADR for several weeks. These data clearly indicate that a cumulative dosage of ADR impairs the cardiovascular functions.

Electron microscopical analysis of the heart samples removed from ADR-treated animals indicated morphological alterations localized in mitochondria. These data are also in agreement with a previous study [34] indicating mitochondrial degeneration induced by ADR. The most pronounced effect of ADR exposure is cumulative dose-dependent mitochondrial damage. However, we observed no apparent myofibrillar disorganization in the heart of ADR-treated animals, as reported by others [35–37]. Although we have no clue as to the reason for this discrepancy, we surmise that it may be due to the different animals and experimental conditions employed. A gradual increase in the number of lipid droplets seen in the heart of mice treated with ADR for 8–9 weeks is also in agreement with other reports [1, 38] and indicative of functional degeneration of the heart tissue. An increased number of lysosomes after treatment with ADR for 12 weeks has also been observed by Singal *et al.* [1], who reported that lysosomal activation appears to be one of the early changes in the rat myocardium in response to ADR treatment (i.p. 15 mg/, cumulative).

The data mentioned thus far strongly suggest that the morphological alterations seen in heart are correlated with electrophysiological dysfunction recorded in ADR-treated animals. In particular, mice treated with ADR for 8 weeks and examined by electron microscopy exhibited some kind of QRS failure as well as an increase in the tQRS value above the control values. These data indicate that monitoring tQRS and QRS failure in anesthetized mice is a useful procedure to evaluate cardiovascular toxicity and cardiomyopathy as proposed earlier with tQRS monitoring in patients treated with ADR [39].

To demonstrate whether the changes seen in the structural and morphological integrity of mitochondria on the one hand and the electrophysiology of the heart in ADR-treated animals on the other are somehow correlated with the effect of ADR on the biosynthesis of COX enzyme, we used Northern blot hybridization analysis. This enabled us to assess the expression of COX genes under the experimental conditions described. By using three cDNA probes corresponding to COXII, COXIII, and COXIV genes and assessing the steady-state level of their RNA transcripts, we observed that among the three genes examined, the mitochondrial COXII gene was the most susceptible to ADR after 8 weeks of treatment. A similar response was observed with liver samples, despite the fact that substantial morphological liver damage was not detected by electron microscopy. The inability of the heart cells to express the COXII gene to normal levels as a result of ADR treatment could impair the biosynthesis and final assembly of the COX holoenzyme, thus apparently leading to inhibition of COX activity [16], to the impairment of mitochondrial function, and finally to depletion of cellular energy supplies. Of course, one could argue that other enzymes involved in the electron transfer chain reaction in mitochondria may also be impaired. The fact, however, that ADR interacts only with the COX enzyme and not with other proteins in mitochondria convinced us to focus on this enzyme [16, 17]. The difference in susceptibility to ADR treatment seen between heart and liver could be due to the different number of mitochondria and energy requirements [40, 41]. The finding that ADR treatment caused no ultrastructural alterations in liver similar to those seen in the cardiac cells is consistent with the selective effect of ADR on heart tissue and the fact that ADR causes no clinical manifestations in liver of patients. Suppression of COXII gene expression by ADR in both heart and liver may have a different impact on these two tissues, considering that heart is far more sensitive to anoxia and energy depletion than liver. The question whether the suppression of COXII gene expression is fully or partially responsible for ADR-induced selective cardiovascular toxicity is still open. What makes the heart more susceptible than liver to ADR treatment may involve more parameters than the impairment of COX enzyme, and needs further documentation.

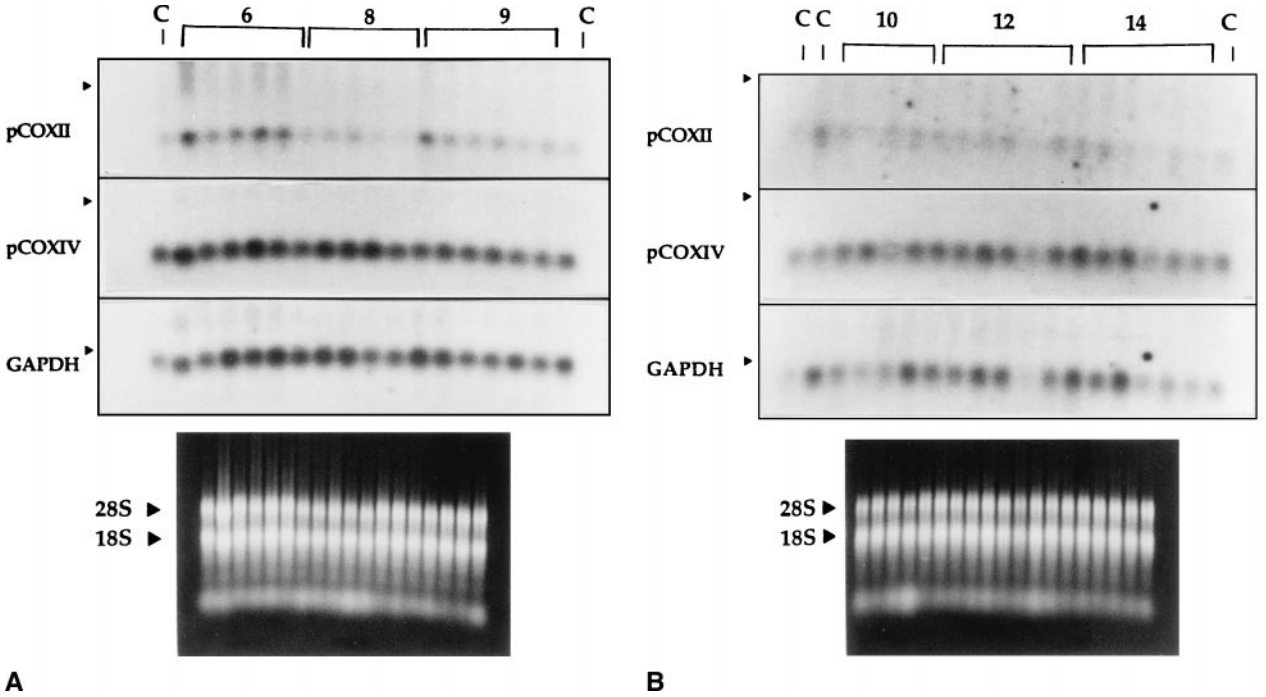

FIG. 7. ADR-induced cumulative dose-dependent effects on COXII and COXIV gene expression in liver tissue. Fifteen μg of RNA samples isolated from liver tissue of control or ADR-treated mice was electrophoretically separated on an agarose–formaldehyde gel, transferred onto a nylon membrane, and hybridized separately with [^{32}P]-labeled probes, exactly as described under Fig. 5. The left panel indicates data from animals treated for 6–9 weeks, the right panel samples obtained from animals treated for 10–14 weeks.

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