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INHIBITION OF MITOCHONDRIAL CARNITINE PALMITOYLTRANSFERASES BY ADRIAMYCIN AND ADRIAMYCIN ANALOGUES

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Abstract—Adriamycin (ADR; doxorubicin) and its highly lipophilic, less toxic analogue *N*-benzyl-adriamycin-14-valerate (AD 198) were found to inhibit rat heart and liver carnitine palmitoyltransferases of both mitochondrial outer and inner membranes. The outer membrane enzyme was more sensitive to inhibition by these drugs than the inner membrane enzyme, and AD 198 was a more potent inhibitor of these enzymes than ADR. Other analogues of ADR, *N*-trifluoroacetyladriamycin-14-valerate (AD 32) and *N*-trifluoroacetyladriamycin-14-*O*-hemiadipate (AD 143), which are documented as being noncardiotoxic, were also more potent inhibitors of the mitochondrial carnitine palmitoyltransferases than ADR. Overall, the cardiac mitochondrial carnitine palmitoyltransferases seemed to be slightly more sensitive to the inhibitory effects of ADR and its analogues than the liver enzyme. ADR was an uncompetitive inhibitor with respect to palmitoyl-CoA and a noncompetitive inhibitor with respect to carnitine for both mitochondrial outer and inner membrane enzymes. Our data suggest that mitochondria can take up ADR and concentrate it within the matrix, as is known to happen with other positively-charged compounds. More ADR was found associated with the mitochondrial inner membrane than with the outer membrane; this could be due to the greater protein content of the inner membrane rather than drug binding to cardiolipin. Although inhibition of cardiac inner membrane carnitine palmitoyltransferase has been implicated previously as part of the cardiotoxicity mechanism of ADR, the present findings with ADR and its noncardiotoxic analogues do not support this view.

The anthracycline glycoside antibiotic adriamycin§ (ADR; doxorubicin) is of major importance in cancer chemotherapy. Unfortunately, the drug shows a cumulative dose-dependent cardiotoxicity, the precise mechanism of which remains unclear. Various mechanisms have been proposed to account for this cardiotoxicity, including the production of cytotoxic free radical species [1, 2], lipid peroxidation of cardiac microsomal membranes [3], differential accumulation and retention of positively-charged drug due to high negative membrane potential [4, 5], release of vasoactive substances [6], and effects on mitochondrial respiration [6, 7]. With regard to this last mechanistic hypothesis, inhibition of the terminal oxidation site of the respiratory chain, cytochrome *c* oxidase, has been suggested to be due to a specific interaction between ADR and cardiolipin of the mitochondrial inner membrane [8]. More recently, it has been suggested that ADR may exert at least a part of its cardiotoxic side effect by inhibiting carnitine palmitoyltransferase [9]. Carnitine palmitoyltransferase (CPT) is an important regulatory enzyme in hepatic fatty acid oxidation. Two CPT activities exist within the mitochondria, one in the mitochondrial inner membrane and one in the mitochondrial outer membrane [10]. The outer membrane enzyme is regulated through inhibition by malonyl-CoA, its physiological inhibitor [11]. Both outer and inner CPT activities were reported to be inhibited by ADR, but the inner membrane enzyme was claimed to be more sensitive to inhibition by ADR because of interactions with cardiolipin [9].

We report here the results of an investigation on the inhibiton of rat heart and liver mitochondrial carnitine palmitoyltransferases by ADR and some of its highly active analogues, *N*-benzyladriamycin-14-valerate (AD 198), *N*-trifluoroacetyladriamycin-14-valerate (AD 32) and *N*-trifluoroacetyladriamycin-14-*O*-hemiadipate (AD 143). These analogues were included in the study because two of them, AD 32 and AD 143, are documented as being noncardiotoxic [12–17]; the third compound, AD 198, is reported to be generally less toxic than ADR [15], although specific *in vivo* cardiotoxicity data for this drug are not yet available.

MATERIALS AND METHODS

Drugs and chemicals. Palmitoyl-CoA, imidazole, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (Hepes), L-carnitine hydrochloride, EDTA, essentially fatty acid-free bovine serum albumin and adriamycin hydrochloride (pure drug without excipient) were purchased from Sigma (St. Louis, MO). *N*-Trifluoroacetyladriamycin-14-valerate, *N*-trifluoroacetyladriamycin-14-*O*-hemiadipate, and *N*-benzyladriamycin-14-valerate hydrochloride were

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§ Adriamycin is a registered trademark of Farmitalia Carlo Erba.

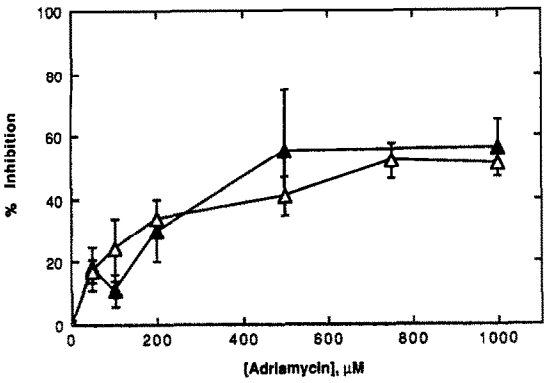

Fig. 1. Comparison of the effects of ADR on hepatic and cardiac carnitine palmitoyltransferases. Intact mitochondria from rat liver (△) and heart (▲) were assayed for the outer carnitine palmitoyltransferase activity as described under Materials and Methods using 40 μM palmitoyl-CoA and 0.5 mM carnitine. Results are means ± SE for three different preparations of mitochondria. Specific activities for the liver and heart enzymes were 9.1 ± 0.4 and 7.1 ± 0.4 nmol/min/mg protein respectively.

synthesized and purified in these laboratories, according to previously described procedures [18–20]. L-[*methyl*-^{3}H]Carnitine hydrochloride was obtained from the Amersham Corp. (Arlington Heights, IL).

Animals. Male Sprague–Dawley rats (180–240 g), obtained from Harlan Industries, Inc. (Indianapolis, IN), were fed Purina Rat Chow (Ralston Purina Co., Richmond, IN) and water *ad lib.* On the day of the experiments, rats were killed by decapitation, and their livers and hearts were removed rapidly for preparation of mitochondria.

Isolation of mitochondria. Intact mitochondria were isolated by the method of Johnson and Lardy [21] with the modifications previously published [22]. The final mitochondrial pellet was resuspended to a concentration of 10 mg/mL in 0.25 M sucrose, 1 mM EDTA, 3 mM Tris, pH 7.2. Protein was determined by the biuret procedure [23].

Mitochondrial outer and inner membrane isolation. To separate outer membranes from mitochondria the method of Parsons *et al.* [24] was used; in this procedure intact mitochondria are suspended in a hypotonic medium to allow the inner membrane to swell to such an extent that the outer membrane is ruptured. Mechanical disruption, followed by centrifugation in sucrose gradients, produced fractions that were rich in outer and inner membranes, as indicated by measurements of the respective marker enzymes, monoamine oxidase and cytochrome *c* oxidase.

Carnitine palmitoyltransferase assay. The method of Bremer [25] was used, as modified and reported previously [26]. Each assay contained, in a total volume of 1 mL: 82 mM sucrose, 70 mM KCl, 35 mM Hepes, 35 mM imidazole, 2 mg bovine serum albumin, L-carnitine (0.4 μCi of L-[*methyl*-^{3}H]carnitine) at the concentrations indicated, 1 μg antimycin A, and palmitoyl-CoA and ADR (or its analogues) at the concentrations indicated.

Inhibition–dilution studies. Intact mitochondria or isolated mitochondrial outer or inner membranes were incubated at 37° with 1 mM ADR for 10 min. An aliquot of this suspension (15 μL in the case of intact mitochondria and mitochondrial inner membrane and 20 μL in the case of the mitochondrial outer membrane) was then assayed for CPT activity in order to determine the amount of inhibition by ADR and whether the inhibition was reversed by dilution (by comparison with assays run in the presence of 1 mM ADR). The remainder of the incubated fractions was then transferred to a 50-mL centrifuge tube and 40 mL of mitochondrial isolating medium [21] was added to the intact mitochondria or, in the case of the inner and outer membranes, 20 mM phosphate buffer, pH 7.2, was added. The resulting suspension was centrifuged at 5600 *g* for the intact mitochondria and 25,000 *g* for the outer and inner membranes. The resulting pellets were then suspended to the original volumes and assayed again for CPT activity.

ADR association with membranes. Intact mitochondria and isolated mitochondrial outer and inner membranes were incubated with 1 mM ADR by mixing 0.9 mL of each preparation with 0.1 mL of a 10 mM ADR solution. The membranes were centrifuged and washed with normal saline, and then the ADR content of each fraction (supernatant solution, wash, and membrane fraction) was measured using reverse phase HPLC, according to methodology published previously [27]. The percentage recovery of the drug was 103 ± 0.2% (mean ± SE of three different preparations of the three membrane fractions, N = 9).

Quenching due to the drugs. ADR and its analogues were extracted along with the radioactive acylcarnitine from CPT assays. These compounds are highly colored and hence quenching in radionuclide quantitation had to be monitored. Various concentrations of the compounds were added to known radioactive standards to determine whether the scintillation counters employed were capable of correcting for the quenching produced. In some experiments, ADR was added after the reaction had been terminated to equalize the concentration in all samples. By this procedure both instruments used in this investigation were found to be able to correct for quenching by ADR and its analogues.

RESULTS

Figure 1 compares the percentage inhibition of intact mitochondrial CPT as a function of ADR concentration for both liver and heart. These data suggest that there is little or no difference between the two different tissues. To see whether there was a difference in inhibition by ADR of the inner CPT of heart compared with liver, the effects of ADR and its analogues on CPT activity were examined in mitochondria that had been frozen and thawed to expose the inner CPT. Table 1 indicates that all three analogues, AD 32, AD 143 and AD 198, inhibited total CPT activity (inner plus outer CPT) more than ADR and that, at a lower concentration of the drugs (0.1 mM), only AD 143 and AD 198 produced greater inhibition of the heart CPT. However, at the

Table 1. Percentage inhibition of frozen and thawed heart and liver carnitine palmitoyltransferases by adriamycin (ADR) and its analogues

Drug concentration (mM)	CPT activity (% inhibition)							
	ADR		AD 32		AD143		AD 198	
	Heart	Liver	Heart	Liver	Heart	Liver	Heart	Liver
0.1	14 ± 7	14 ± 8	29 ± 15	22 ± 12	31 ± 10	11 ± 4	56 ± 23	37 ± 3
1	70 ± 7	38 ± 6	100	61 ± 6	100	70 ± 6	100	100

Frozen and thawed mitochondria from rat liver and heart were assayed for total carnitine palmitoyltransferase (CPT) activity as described in Materials and Methods using 40 μM palmitoyl-CoA and 0.5 mM carnitine. Results are mean percentages ± SE for three different preparations of mitochondria. Specific activities for the liver and heart enzymes were 10.6 ± 0.5 and 7.4 ± 0.9 nmol/min/mg protein respectively.

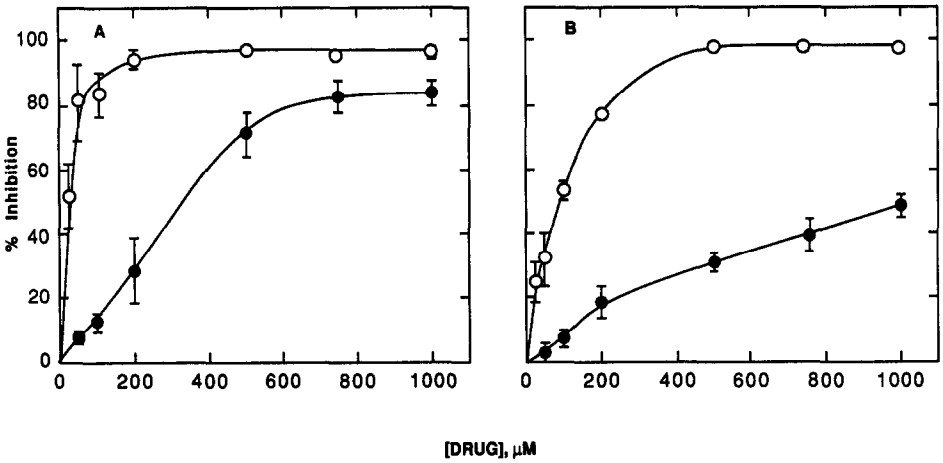

Fig. 2. Inhibition of carnitine palmitoyltransferases by ADR and AD 198. Isolated outer (A) and inner (B) hepatic mitochondrial membranes were assayed for carnitine palmitoyltransferase activity as described under Materials and Methods using 40 μM palmitoyl-CoA and 0.5 mM carnitine. The effects of ADR (●) and AD 198 (○) are presented as means ± SE for three different preparations of membranes. The specific activities of the isolated mitochondrial outer and inner membrane enzymes were 26.0 ± 2 and 14.3 ± 0.8 nmol/min/mg protein respectively.

higher concentration of the drugs (1.0 mM), heart mitochondrial CPT was more sensitive than liver mitochondrial CPT for ADR and all three analogues.

Figure 2 demonstrates that the outer membrane CPT was more sensitive to inhibition by ADR and AD 198 than the inner membrane enzyme. AD 198 was a much more potent inhibitor of both enzymes, affording I_{50} values that were approximately 10-fold lower than ADR.

Figure 3 compares the activity of the rat liver mitochondrial outer membrane CPT in the presence and absence of ADR as a function of palmitoyl-CoA concentration. Both the V_{max} of CPT and the K_m for palmitoyl-CoA were decreased by ADR, indicating that the type of inhibition exhibited by the drug is uncompetitive, rather than noncompetitive. To confirm the uncompetitive inhibition by ADR, Dixon plots [28] for both outer and inner membrane CPT were constructed. Figure 4 indicates that the inhibition observed due to ADR with respect to palmitoyl-CoA was uncompetitive in nature for both the outer and inner membrane enzymes. Figure 5 indicates that the inhibition exhibited by ADR with respect to carnitine was noncompetitive in nature for both outer and inner membrane CPT. These results were also confirmed by Cornish–Bowden plots [29] (data not shown). Brady and Brady [9] reported that the inhibition by ADR is noncompetitive with respect to both L-carnitine and palmitoyl-CoA; however, their data were not shown and thus could not be analyzed.

While incubating mitochondria with ADR for studies of inhibition of CPT, we observed that, when the ADR was added to the intact mitochondria, after a few seconds the mitochondria precipitated out. Upon precipitation, the mitochondria retained much of the color of the ADR i.e. the adriamycin seemed to be trapped or concentrated in the mitochondrial precipitate. On addition of Triton X-100, the precipitated mitochondria were dispersed and the color

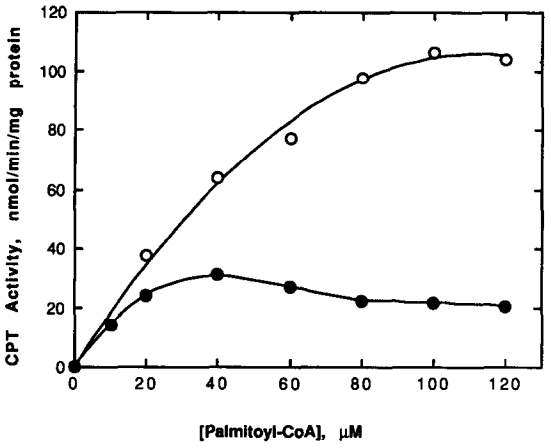

Fig. 3. Inhibitory effect of ADR on rat liver mitochondrial outer membrane carnitine palmitoyltransferase. Inhibition by 300 μM ADR (●) was compared with activity in the absence of ADR (○). Mitochondrial outer membrane carnitine palmitoyltransferase was assayed at a constant palmitoyl-CoA to albumin ratio (3:1) and 0.5 mM carnitine. Other conditions are as described under Materials and Methods. Results are representative of three different preparations of mitochondrial outer membranes.

of the incubate became uniform. With mitochondrial inner membranes, this effect was also observed but to a much lesser extent; with the mitochondrial outer membranes, the effect was not evident. These observations suggest that ADR may become concentrated either within the matrix space or in the mitochondrial membranes. Because of these observations, the effects of dilution and washing of the mitochondria, and of the isolated membranes, on inhibition of CPT by ADR were examined. Experiments designed to assess the degree of association of ADR with the various fractions were also conducted.

When intact mitochondria, and isolated outer and inner membranes, were incubated with 1 mM ADR for 10 min and aliquots of these incubation mixtures assayed for CPT activity, the same extent of inhibition was found upon dilution to be maintained by both the intact mitochondria and inner membranes, whereas the inhibition of the outer membrane CPT was lost (Fig. 6). These results suggest that inhibition in the outer membranes was essentially reversed, but that in inner membranes and intact mitochondria inhibition was retained. The retention of inhibition of the inner CPT could be due to irreversibility, binding of ADR to membranes or trapping of ADR in the mitochondrial matrix. Upon resuspension of the various incubated fractions, the CPT activity returned to that of the control, which, in the case of the intact mitochondria or inner membranes, would indicate that ADR inhibition was reversible and simply needed more vigorous mechanical manipulation or a greater dilution gradient to allow the inhibitor to be released. In contrast, Brady and Brady [9] reported that no inhibition was seen when an aliquot of mitochondria or vesicles that had been preincubated with ADR was assayed for CPT activity. Inhibition–dilution studies with AD 198 indicated that dilution resulted in partial restoration of CPT activity in the intact mitochondria, presumably because AD 198 is not as positively charged at neutral pH as ADR (data not shown).

Since ADR inhibited CPT of the mitochondrial outer membrane more than CPT of the mitochondrial inner membrane, and because Brady and Brady have suggested that there should be more ADR associated with the mitochondrial inner membrane due to its greater content of cardiolipin, it was necessary to know how much ADR was associated with each membrane fraction. When mitochondria were incubated with 1 mM ADR in a total volume of 1 mL and then washed to remove extramitochondrial drug, it was found that the intact mitochondria took up and retained about 60% of the ADR added (Table

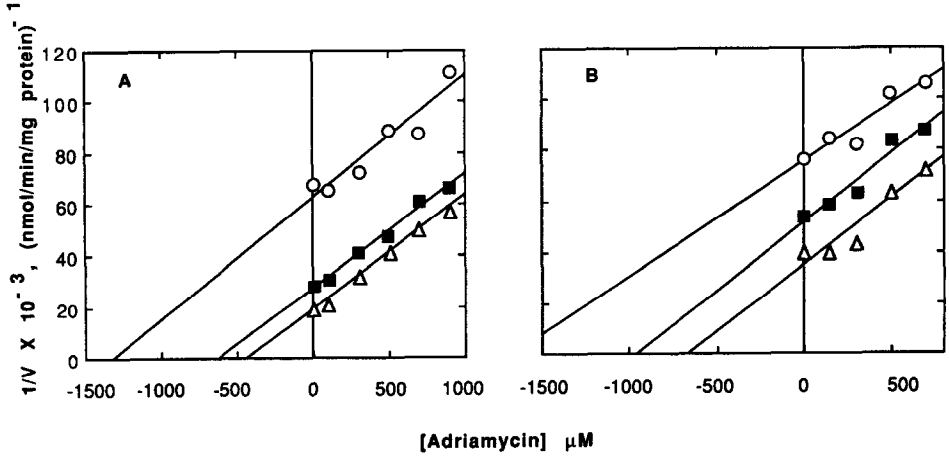

Fig. 4. Dixon plot for inhibition with respect to palmitoyl-CoA of mitochondrial outer (A) and inner (B) membrane carnitine palmitoyltransferases by ADR. Isolated rat liver mitochondrial outer and inner membranes were assayed as described under Materials and Methods using (panel A) 10 μM (○), 20 μM (■), and 30 μM (△) palmitoyl-CoA for the outer membranes at a carnitine concentration of 0.5 mM, and (panel B) 25 μM (○), 40 μM (■), and 80 μM (△) palmitoyl-CoA for the inner membranes at a carnitine concentration of 1.0 mM. Results are representative of three different membrane preparations.

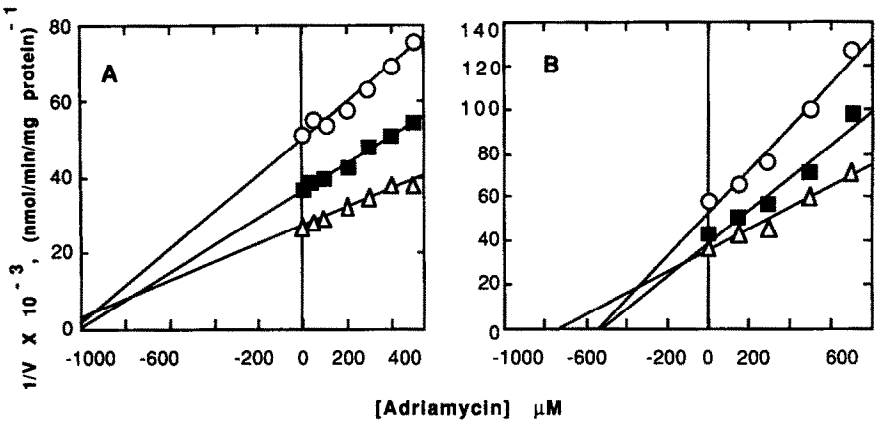

Fig. 5. Dixon plot for inhibition with respect to carnitine of mitochondrial outer (A) and inner (B) membrane carnitine palmitoyltransferases by ADR. Isolated rat liver mitochondrial outer and inner membranes were assayed as described under Materials and Methods using (panel A) 0.1 mM (○), 0.2 mM (■), and 0.5 mM (△) carnitine for outer membranes at a palmitoyl-CoA concentration of 60 μM and (panel B) 0.25 mM (○), 0.5 mM (■), and 1.0 mM (△) carnitine for inner membranes at a palmitoyl-CoA concentration of 100 μM. Results are representative of three different membrane preparations.

2). Less ADR was associated with the inner membranes and even less with the outer membranes; however, from the known protein concentration added and the amount of ADR added and recovered in each fraction, it was calculated that ADR was associated with each membrane fraction in approximate proportion with the amount of protein present. These data suggest that binding of ADR to protein is the most important factor determining its association with these membranes.

DISCUSSION

Despite the major importance of ADR in cancer chemotherapy, its use is limited in part by its potential cardiotoxicity. It has been reported that L-carnitine may protect against ADR-induced cardiotoxicity by interfering with the formation of ADR–cardiolipin complex [30]. Since L-carnitine is a substrate for CPT, this finding suggests that CPT may also be involved in this cardiotoxicity. In this regard, Brady and Brady [9] have indicated a preferential inhibition of mitochondrial inner membrane CPT relative to that of the outer membrane CPT by ADR, and they have suggested that inhibition of cardiac CPT by ADR may be important in producing cardiotoxicity.

The concentration range of ADR used in these studies was comparable with that employed by Brady and Brady [9] in their work on ADR inhibition of hepatic and cardiac CPT and does not reflect tissue concentration of this drug achieved following therapeutic dosing in animals. The data presented here, comparing the sensitivities of intact mitochondrial carnitine palmitoyltransferases to ADR are in contrast to the data presented by Brady and Brady [9] which suggested that the heart outer mitochondrial CPT was less sensitive to inhibition by ADR than liver outer mitochondrial CPT. This discrepancy could be due to the way that the mitochondria were prepared since Brady and Brady have used Nagarse in their preparations of heart mitochondria. It has been reported by Murthy and Pande [10] that, although exposure to proteases (including Nagarse) does not affect the outer membrane CPT activity, the inhibitory effects of malonyl-CoA are decreased greatly. It could be that exposure to Nagarse also decreases the sensitivity of outer membrane CPT to ADR.

The sensitivity of the outer membrane CPT to inhibition by ADR and AD 198 was greater than that of the inner membrane enzyme. For ADR these results are in contrast to data presented by Brady and

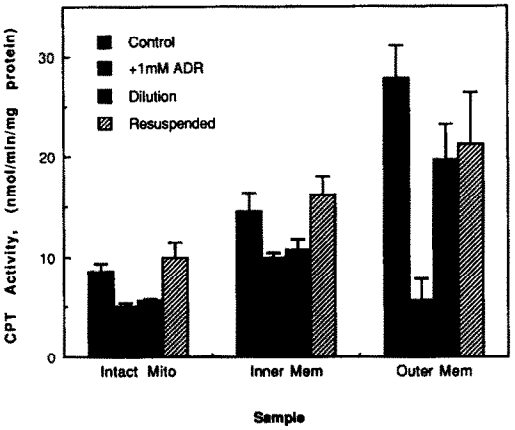

Fig. 6. Incubation-dilution experiments. Intact mitochondria, and isolated inner and outer membranes were assayed for carnitine palmitoyltransferase activity as described under Materials and Methods using 40 μM palmitoyl-CoA and 0.5 mM carnitine. Results are means ± SE of five, three and four different preparations of intact mitochondria, and isolated inner and outer membranes respectively.

Table 2. Distribution of adriamycin (ADR) in hepatic mitochondrial membranes

Membrane type	Membrane fraction (%)	Supernatant + wash (%)	Membrane protein (mg/mL)	ADR content (μg/mg protein)
Intact mitochondria	61.2 ± 1.2	38.8 ± 1.2	6.5 ± 0.8	70 ± 9
Inner membrane	32.4 ± 3.2	67.6 ± 3.2	3.0 ± 0.4	79 ± 7
Outer membrane	11.3 ± 1.3	89.7 ± 1.3	1.4 ± 0.1	60 ± 10

ADR (1 mM, total volume of 1 mL) was incubated with intact mitochondria and with isolated mitochondrial inner and outer membranes; then the mitochondrial fractions were washed and analyzed for ADR as described in Materials and Methods. The tabulated data indicate the percentage of applied ADR recovered in the membrane fraction, the percentage of applied ADR not associated with the membrane (wash + supernatant), and the amount of ADR associated with each membrane fraction per mg of membrane protein. Values are means ± SE for three different preparations.

Brady [9], who reported that the intact mitochondrial CPT is less sensitive to inhibition by ADR than CPT in inverted submitochondrial vesicles. Again, the differences in our results could be due to the way in which the inner membranes were prepared. Brady and Brady have studied the inner CPT by preparing inverted submitochondrial vesicles. This method gives single-layered vesicles of membranes which are inside-out. The method we used produces unbroken inner membranes. Both of these methods can produce inner membranes that are contaminated with outer membranes, but apparently there is some difference in the inner membrane itself, possibly due to the spatial orientation of the membrane.

Kinetic data obtained here on the inhibition of carnitine palmitoyltransferases indicate that the inhibition is uncompetitive with respect to palmitoyl-CoA and noncompetitive with respect to carnitine for both mitochondrial outer and inner membrane respectively. This type of inhibition is quite different from that observed with other inhibitors of CPT, such as malonyl-CoA and tetradecylglycidic acid, which inhibit in the form of the coenzyme A ester [31, 32], and the pro-drug oxfenicine, which must be transaminated to the free carboxylic acid form before inhibiting [33]. Malonyl-CoA is a competitive inhibitor with respect to palmitoyl-CoA and is noncompetitive with respect to carnitine. The metabolite of oxfenicine, hydroxyphenylglyoxylate, is competitive with respect to carnitine and noncompetitive with respect to palmitoyl-CoA [33]. The sulfonylureas are noncompetitive with respect to palmitoyl-CoA and uncompetitive with respect to carnitine [34], the latter mode of inhibition being opposite to that observed with ADR. Uncompetitive inhibition by sulfonylureas implies that the drug binds to the enzyme–carnitine complex, whereas with ADR the drug apparently binds to the enzyme–palmitoyl-CoA complex. The significance of this is not clear as yet; however, it suggests that the enzyme must have different binding sites for these various inhibitors since it would be unlikely for such a different array of molecules to bind to the same site and produce completely different types of inhibition.

It has been suggested that ADR added to whole mitochondria becomes associated mainly with the mitochondrial inner membrane because of binding to cardiolipin [35]. Brady and Brady have reported that the mitochondrial inner membrane CPT is more sensitive to inhibition by ADR and suggested that the presence of cardiolipin in the inner membrane enhances the inhibition of CPT [9]. Other important factors to be considered in this regard are the facts that ADR binds to protein [36] and that the mitochondrial inner membrane contains more protein than the other cellular membranes [37]. In contrast, the mitochondrial outer membrane contains much less protein than the average cell membrane [37]. Results presented here indicate that ADR is associated with the mitochondrial inner and outer membranes in approximate proportion to the protein content of the membrane, thus suggesting that ADR binds to the protein component rather than the lipid component of the membrane. Furthermore, the present data indicate that the CPT of isolated outer membranes is more sensitive to inhibition by ADR than any other CPT activity, even though the outer membrane contains less cardiolipin than the inner membrane [38]. These observations further suggest that the amount of ADR bound to the membrane may not be related to the amount of inhibition produced. Since other inhibitors of CPT, such as malonyl-CoA [22] and the hypoglycemic sulfonylureas [34], also display a greater ability to inhibit the outer membrane CPT compared with the inner membrane CPT, it is suggested that inhibition by ADR depends on specific binding to the CPT protein, which is somewhat different for the two enzymes.

The ADR analogues AD 32 and AD 143 exhibit high antitumor activity with essentially no evidence of cardiac toxicity in laboratory animal model systems [12–15] and in humans [16, 17]. The availability of these agents made possible their use as probes in evaluating the relationship between anthracycline-induced cardiotoxicity and CPT inhibition. These compounds proved to be more potent inhibitors than ADR for all CPT activities tested. While the concentration of ADR needed to inhibit CPT activities may be high relative to tissue levels achieved *in vivo* following the chronic administration of therapeutically-active and cardiotoxic doses to animals, the concentrations of the noncardiotoxic analogues are fully consistent with tissue levels achieved *in vivo* with therapeutic doses [39]. Accordingly, it must be concluded that the unusual cardiotoxicity of ADR does not appear to be related in any way to its

inhibition of CPT. Data obtained in this study indicate that the ADR analogue AD 198 is an even more potent inhibitor of the mitochondrial outer and inner membrane CPT than AD 32 or AD 143. Since AD 198 has been reported to be generally less toxic than ADR [15], it will be interesting to see whether it also has lower cardiotoxic potential. Murine cardiotoxicity assays with this agent are currently in progress.

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Note added in proof. The cardiotoxic evaluation of AD 198, utilizing a well-accepted murine model of anthracycline-induced cardiac toxicity (Bertazzoli C, Bellini O, Magrini U and Tosana MG, Quantitative experimental evaluation of adriamycin cardiotoxicity in the mouse. *Cancer Treat Rep* **63**: 1877–1883, 1979), has now been completed. Based on a histopathology scoring system which factors the severity and extent of myocardial damage in treated animals, the results show AD 198 indeed to be markedly less cardiotoxic than ADR (Israel M, Koseki Y and Jenkins JJ III, unpublished observations), further supporting the conclusions of this present report. Complete details on the comparative cardiotoxicity evaluation of AD 198 and ADR will be presented elsewhere.

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