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PROPIONYL-L-CARNITINE AS POTENTIAL PROTECTIVE AGENT AGAINST ADRIAMYCIN-INDUCED IMPAIRMENT OF FATTY ACID BETA-OXIDATION IN ISOLATED HEART MITOCHONDRIA

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Propionyl-L-carnitine (PLC), a natural short-chain derivative of L-carnitine, has been tested in this study as a potential protective agent against adriamycin (ADR)-induced cardiotoxicity in isolated rat heart myocytes and mitochondria. In cardiac myocytes, ADR (0.5 mM) caused a significant (70%) inhibition of palmitate oxidation, whereas, PLC (5 mM) induced a significant (49%) stimulation. Addition of PLC to ADR-incubated myocytes induced 79% reversal of ADR-induced inhibition of palmitate oxidation. In isolated rat heart mitochondria, ADR produced concentration-dependent inhibition of both palmitoyl-CoA and palmitoyl-carnitine oxidation, while PLC caused a more than 2.5-fold increase in both substrates. Preincubation of mitochondria with 5 mM PLC caused complete reversal of ADR-induced inhibition in the oxidation of both substrates. Also ADR induced concentration-dependent inhibition of CPT I which is parallel to the inhibition of its substrate palmitoyl-CoA. In rat heart slices, ADR induced a significant (65%) decrease in adenosine triphosphate (ATP) and this effect is reduced to 17% only by PLC. Results of this study revealed that ADR induced its cardiotoxicity by inhibition of CPT I and β-oxidation of long-chain fatty acids with the consequent depletion of ATP in cardiac tissues, and that PLC can be used as a protective agent against ADR-induced cardiotoxicity.

KEY WORDS: adriamycin, beta-oxidation, cardiotoxicity, propionyl-L-carnitine.

INTRODUCTION

The anthracycline glycoside antibiotic adriamycin (ADR) is of major importance in cancer chemotherapy [1]. However, both patients and experimental studies exhibit a cumulative dose-dependent and irreversible cardiotoxicity which limit its usefulness as a broad spectrum anticancer drug [2, 3]. Although the precise mechanism of this pathogenesis is not yet completely known [4, 5], it has been suggested that the inhibition of long-chain fatty acid oxidation in the heart by ADR is an important mechanism in the development of ADR-related cardiotoxicity [6, 7].

Since long-chain fatty acids are the major substrates for energy production in the aerobic adult myocardium [8], their inhibition is usually associated with cardiomyopathy and congestive heart failure due to deficiency in energy supply and accumulation of their toxic intermediates in cardiac tissues [9]. Although alteration of fatty acid oxidation has been associated with ADR use, the exact site of inhibition remains to be determined. In an attempt to reduce and/or control ADR-induced cardiotoxicity, a number of drugs have been examined depending on the underlying mechanism of cardiotoxicity [10–12]. A recent study in our laboratory demonstrated that L-carnitine partially protects the myocardium against ADR-induced cardiotoxicity without interfering with its antitumour activities [13]. Propionyl-L-carnitine (PLC) is a natural short-chain derivative of L-carnitine and it has a higher transport rate into the myocardium than L-carnitine [14]. Also, it has been suggested that PLC has a more pronounced protective effect than L-carnitine in some models of

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ischaemia and cardiomyopathies, but again no mechanism for this effect has been ascertained [15]. Therefore, this study has been initiated to determine the effect of ADR and/or PLC on the oxidation of long-chain fatty acid in isolated cardiac cells and mitochondria with the following specific aims: (i) determination of the exact site of inhibition of long-chain fatty acid oxidation by ADR; and (ii) identifying the mechanism whereby PLC could reverse ADR-induced inhibition of fatty acid oxidation.

MATERIAL AND METHODS

Animals

Male Sprague–Dawley rats, weighing 200–250 g were obtained from the animal house of the National Cancer Institute (NCI) Cairo University. Animals were allowed free access to standard diet essentially free from L-carnitine derivatives and water *ad libitum*.

Materials

[1-^{14}C]Palmitate, [1-^{14}C]palmitoyl-CoA, [methyl-^{14}C]L-carnitine and [1-^{14}C]palmitoyl-carnitine were purchased from New England Nuclear (Boston, MA, USA). Sigma was the source of bovine serum albumin (BSA, essentially fatty acid free), palmitoyl-carnitine and palmitoyl-CoA. ADR was a generous gift from NCI drug store. PLC was a generous gift from Dr Salah Abdel-aleem (Duke University, Medical Center, NC, USA). Collagenase type II was purchased from Worthington (NJ, USA), and Joklik essential medium was purchased from Gibco laboratories (NJ, USA).

Isolation of cardiac myocytes

Adult rat heart myocytes were isolated according to the method of Frangakis *et al.* [16]. Myocytes were isolated with Joklik essential medium containing 5.55 mM glucose, 25 mM $NaHCO_3$, 1.2 mM $MgSO_4$ and 0.5 mM $CaCl_2$ (pH 7.4). The viability of myocytes isolated by this procedure was 80–90% as determined by trypan blue dye exclusion test.

Palmitate oxidation in myocytes

Myocytes (2 mg cell protein) suspended in 0.9 ml of Joklik medium, containing 25 mM $NaHCO_3$, 5.55 mM glucose, 1.2 mg $MgSO_4$, 0.5 mM $CaCl_2$ and 10 mM HEPES (pH 7.4), were placed in a 25-ml Erlenmeyer flask. Cells were preincubated with ADR (0.5 mM) and/or PLC (5 mM) for 10 min at 37°C under constant shaking. To this cell suspension 0.1 ml of 2 mM [1-^{14}C]palmitic acid was added yielding a final concentration of 0.2 mM [1-^{14}C]palmitic acid (2.2×10^5 dpm). The Erlenmeyer flask was then closed with a rubber septum to which a plastic centre well was attached. The incubation was continued under shaking at 37°C for 30 min. An injection of 0.3 ml of 1 M hyamine hydroxide was administered through the septum into the centre well to absorb the released CO_2, and the reaction was terminated by injecting 1 ml of 7% perchloric acid through the septum into the incubation medium. The flasks were shaken continuously for an additional 2 h at 37°C. After that time, the plastic centre well was removed, placed into a scintillation vial containing 10 ml of Scinti Verse BD, and counted in a liquid Scintillation Counter (Betamatic Kontron, Sebai, Italy).

Isolation of rat heart mitochondria

Rat heart mitochondria were isolated by the procedure of Chappel and Hansford [17]. The isolation buffer contained 0.21 M mannitol, 0.07 M sucrose, 5 mM Tris–HCl (pH 7.4), and 1 mM EDTA.

Oxidation of palmitoyl-CoA and palmitoyl-carnitine in rat heart mitochondria

Substrate oxidation in mitochondria was measured according to the method of Yang *et al.* [18]. The reaction mixture contained in a final volume of 1.0 ml, 50 mM Tris–HCl (pH 7.4); 120 mM KCl; 0.5 mM L-carnitine and 0.5 mM EDTA-K_2 (pH 7.4), 2 mM KP_i, and 0.1 mg ml^{-1} BSA, 50 μM [1-^{14}C]palmitoyl-carnitine or 40 μM [1-^{14}C]palmitoyl-CoA, were placed in a 25-ml Erlenmeyer flask. Substrate oxidation was initiated by the addition of rat heart mitochondria (0.5–1 mg) which were preincubated with saline (control), ADR and/or PLC for 10 min at 37°C. The rate of oxidation of palmitoyl-carnitine and palmitoyl-CoA was determined using the same procedure described before for measuring the release of $^{14}CO_2$ with myocytes.

Assay of carnitine palmitoyltransferase enzyme

The activity of outer carnitine palmitoyltransferase (CPT I) was measured in intact mitochondria according to Kashfi *et al.* [19]. The assay depends on the formation of palmitoyl-[methyl-^{14}C]L-carnitine using [methyl-^{14}C]L-carnitine. In brief, the reaction mixture in a total volume of 1 ml contained 82 mM sucrose, 70 mM KCl, 35 mM hepes, 35 mM imidazole, 2 mg BSA, 40 μM palmitoyl-CoA, 0.5 mM (0.4 μCi of [methyl-^{14}C]L-carnitine and ADR at the concentration indicated. The reaction was initiated by addition of mitochondria (0.5–1 mg) for 10 min and then terminated by injecting 1 ml of 7% perchloric acid. Then, the formed palmitoyl-[methyl-^{14}C]L-carnitine was extracted three times with water-saturated butanol. An aliquot of 0.5 ml of butanol extract was placed into a scintillation vial containing 10 ml of Scinti Verse BD, and counted in a liquid scintillation counter.

Quenching due to ADR

ADR was extracted along with the radioactive

palmitoyl-L-carnitine during CPT I assays. ADR is highly coloured and hence quenching in radionuclide quantitation had to be monitored. Various concentrations of ADR were added to known radioactive standards to determine whether the scintillation counter employed was 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.

Assessment of adenosine triphosphate

Tissue ATP contents were determined in rat heart slices according to Neri *et al.* [20]. Heart slices were incubated for 60 min at 37°C with ADR (14 μg ml^{-1}), PLC (600 μg ml^{-1}), ADR plus PLC, and saline (control). Tissue ATP contents were determined using high-pressure liquid chromatography (Konttron, 322, Sebai, Italy).

Determination of protein

Protein concentration were determined by Bio-Rad protein assay (Bio-Rad, Richmond, VA, USA) according to the method of Bradford [21].

RESULTS

Figure 1 shows the effect of ADR (0.5 mM), PLC (5 mM), and their combination on [1-^{14}C]palmitate oxidation in isolated cardiac myocytes. After a 10-min preincubation with myocytes, ADR caused a significant (70%) inhibition of palmitate oxidation. Whereas, PLC induced significant 49% increase in palmitate oxidation. The addition of PLC to ADR-incubated myocytes resulted in 79% recovery of ADR-induced inhibition of palmitate oxidation.

The effects of ADR (0.5 mM), PLC (5 mM) and their combination on [1-^{14}C]palmitoyl-CoA oxidation in rat heart mitochondria as a function of incubation time are shown in Table I. ADR induced a significant (45%) decrease, while PLC caused highly significant (230%) increase of palmitoyl-CoA oxidation in a time-dependent manner. Preincubation of mitochondria with PLC resulted in 100% recovery of ADR-induced inhibition of palmitoyl-CoA oxidation and a 65% increase compared to control mitochondria.

Figure 2 shows dose–response curve for the effect of ADR (0.05–2 mM) alone and in combination with PLC (5 mM) on [1-^{14}C]palmitoyl-CoA oxidation in isolated rat heart mitochondria. The oxidation of palmitoyl-CoA was significantly decreased by ADR alone in a concentration-dependent manner. However, the addition of PLC to ADR-incubated mitochondria resulted in complete reversal of ADR-induced inhibition of palmityol-CoA oxidation except at a higher concentration of ADR (2 mM).

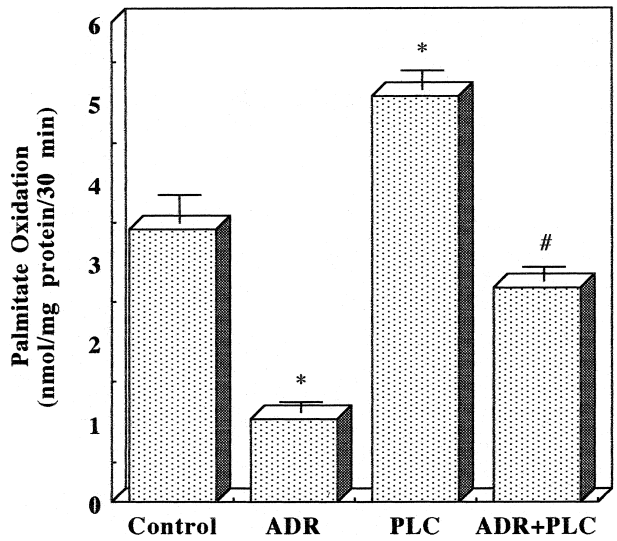

Fig. 1. Effect of ADR, PLC and their combination on palmitate oxidation in isolated cardiac myocytes. (1) Values are presented as mean ± SD of at least four separate experiments. (2) * Indicates significant change of ADR and PLC *vs* control ($P < 0.05$). (3) # Indicate significant change of ADR + PLC *vs* ADR ($P < 0.05$).

The effect of different concentrations of ADR (0.05–2 mM) alone and in the presence of PLC (5 mM) on palmitoyl-carnitine oxidation in rat heart mitochondria are shown in (Fig. 3). In the absence of PLC, ADR induced a significant concentration-dependent inhibition, while the presence of PLC resulted in complete recovery of ADR-induced inhibition of palmitoyl-carnitine oxidation.

Table II shows the effect of different concentrations of ADR (0.05–2 mM) on CPT I in isolated mitochondria. ADR caused concentration-dependent inhibition of CPT I which is parallel to the inhibition of its substrate palmitoyl-CoA.

The effects of ADR (14 μg ml^{-1}) and PLC (600 μg ml^{-1}) and their combination on ATP contents in

Table I
Effect of ADR, PLC and their combination on palmitoyl-CoA oxidation in isolated rat heart mitochondria

Addition	*Palmitoyl-CoA oxidation nmol mg^{-1} protein^{-1}*		
	15 min^{-1}	*30 min^{-1}*	*45 min^{-1}*
None	3.4 ± 0.5	7.6 ± 0.7	11.1 ± 1.0
ADR (0.5 mM)	2.1 ± 0.1*	4.3 ± 0.5*	6.3 ± 0.4*
PLC (5 mM)	8.1 ± 0.6*	17.3 ± 1.3*	26.0 ± 0.9*
ADR + PLC	5.5 ± 0.4*†	11.8 ± 1.3*†	19.1 ± 1.3*†

Notes. Values are presented as mean ± SD of at least four separate experiments. * Indicates significant change of ADR, PLC, and ADR + PLC *vs* control ($P < 0.05$); †indicates significant change of ADR + PLC *vs* ADR ($P < 0.05$).

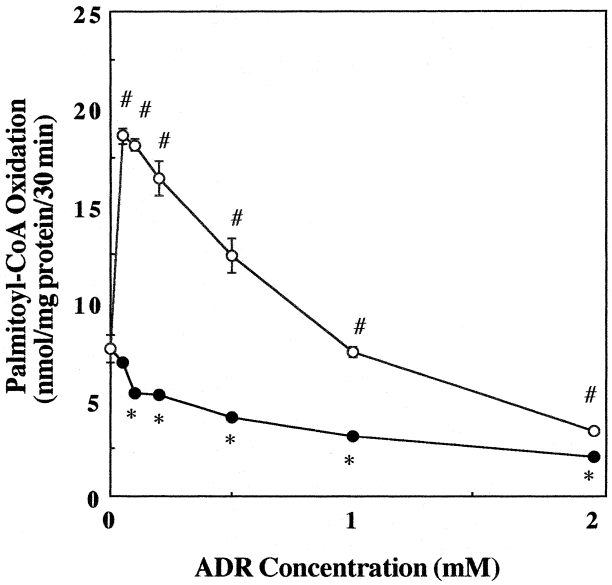

Fig. 2. Dose–response curve of the effect of ADR (●) and ADR + PLC (○) on palmitoyl-CoA oxidation in isolated rat heart mitochondria. (1) Values are presented as mean ± SD of at least four separate experiments. (2) *Indicates significant change of ADR *vs* control ($P < 0.05$). (3) #Indicates significant change of ADR + PLC *vs* ADR ($P < 0.05$).

rat heart slices are shown in Table III. ADR induced a significant (65%) decrease in ATP and this effect is reduced to 17% only by PLC.

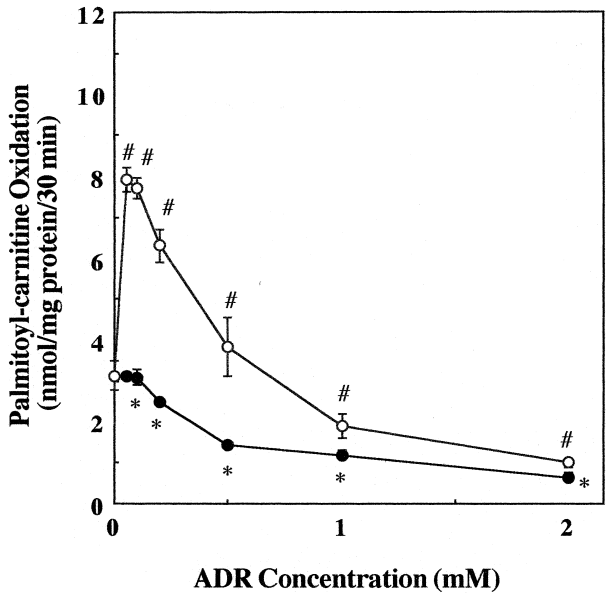

Fig. 3. Dose–response curve of the effect of ADR (●) and ADR + PLC (○) on palmitoyl-carnitine oxidation in isolated rat heart mitochondria. (1) Values are presented as mean ± SD of at least four separate experiments. (2) *Indicates significant change of ADR *vs* control ($P < 0.05$). (3) #Indicates significant change of ADR + PLC *vs* ADR ($P < 0.05$).

Table II
Dose–response curve of the effect of ADR on carnitine palmitoyltransferase in isolated rat heart mitochondria

Concentration of ADR (mM)	*Carnitine palmitoyl transferase activity*	
	nmol mg protein^{-1} min^{-1}	*% inhibition*
0.0	16.19 ± 0.88	0
0.05	15.29 ± 1.81	6
0.1	13.20 ± 1.17*	18
0.2	12.65 ± 1.04*	22
0.5	12.22 ± 1.32*	25
1.0	9.32 ± 1.98*	42
2.0	8.25 ± 0.72*	49

Notes. Values are presented as mean ± SD of at least four separate experiments. *Indicates significant change of ADR *vs* control ($P < 0.05$).

DISCUSSION

Under normal physiological conditions, palmitate oxidation supply the heart with 70% of ATP and the remaining 30% of ATP is supplied via glucose, lactate and ketone bodies utilization [8]. Therefore, inhibition of palmitate oxidation in the heart is associated with cardiotoxicity due to a deficiency in ATP supply and accumulation of toxic palmitoyl-CoA and palmitoyl-carnitine [9]. Drugs which increase ATP production and prevent the accumulation of toxic fatty acid intermediates in the heart become potential candidates in the treatment of cardiotoxicity. In our laboratory, we reported that L-carnitine partially protects the heart against ADR-related cardiotoxicity in a dose-dependent manner [13]. Data reported in this study proved that PLC completely protects against ADR-induced cardiotoxicity. This effect may be due to the increase in the intracellular concentration of L-carnitine and the flux of acetyl-CoA through the Kreb's cycle.

Our data (Fig. 1) suggest that PLC stimulates palmitate oxidation and reverses the ADR-induced inhibition of palmitate oxidation in isolated cardiac myocytes. This effect could occur as a secondary event following PLC metabolism (Fig. 4), since PLC can easily be transported into cardiac myocytes

Table III
Effect of ADR, PLC and their combination on adenosine triphosphate contents in rat heart slices

Adenosine	*Adenosine triphosphate nmol mg protein^{-1}*
None	35.90 ± 2.80
ADR (14 μg ml^{-1})	12.56 ± 1.71*
PLC (600 μg ml^{-1})	33.02 ± 3.46
ADR + PLC	29.82 ± 3.26†

Notes. Values are presented as mean ± SD of ten determinations after 60-min incubations. *Indicates significant change of ADR *vs* control ($P < 0.05$); †indicates significant change of ADR + PLC *vs* ADR ($P < 0.05$).

through sarcolemmal carnitine carriers and within the myocytes into mitochondria through carnitine/acylcarnitine translocase (CT) [14]. In mitochondria, PLC has a high affinity for CoA-SH:carnitine acetyltransferase and being converted into free L-carnitine and propionyl-CoA [15]. The released L-carnitine may increase the oxidation of palmitate by increasing its mitochondrial transport through CPT I and/or the increase in mitochondrial CoA-SH/acetyl-CoA ratio. Previously, Sayed-Ahmed [22] and Abdel-aleem *et al.* [23] reported that L-carnitine increased the mitochondrial efflux of pyruvate-generated acetyl-CoA in the form of acetyl carnitine in a reaction mediated by carnitine acetyl transferase enzyme (CAT) (Fig. 4). The propionyl-CoA formed in mitochondria due to PLC metabolism may also stimulate palmitate oxidation since it can be converted into succinyl-CoA in a reaction mediated by propionyl-CoA carboxylase [24], thus increasing with an anaplerotic process, the flux of acetyl-CoA through the Kreb's cycle (Fig. 4). As a result of these two opposite effects of PLC: the increased mitochondrial efflux of carbohydrate-generated acetyl-CoA by the L-carnitine portion of PLC [22, 23], and the increase in acetyl-CoA flux through the Kreb's cycle (anaplerotic reaction) by propionate portion of PLC [24], carbohydrate oxidation should be unchanged. This has been confirmed by the recent data presented by Soloma [25] which demonstrated that although PLC increased palmitate oxidation and has no effect on both [1-^{14}C]pyruvate oxidation (an index of pyruvate dehydrogenase activity) and [2-^{14}C]pyruvate oxidation (an index of acetyl-CoA flux through Kreb's cycle), the

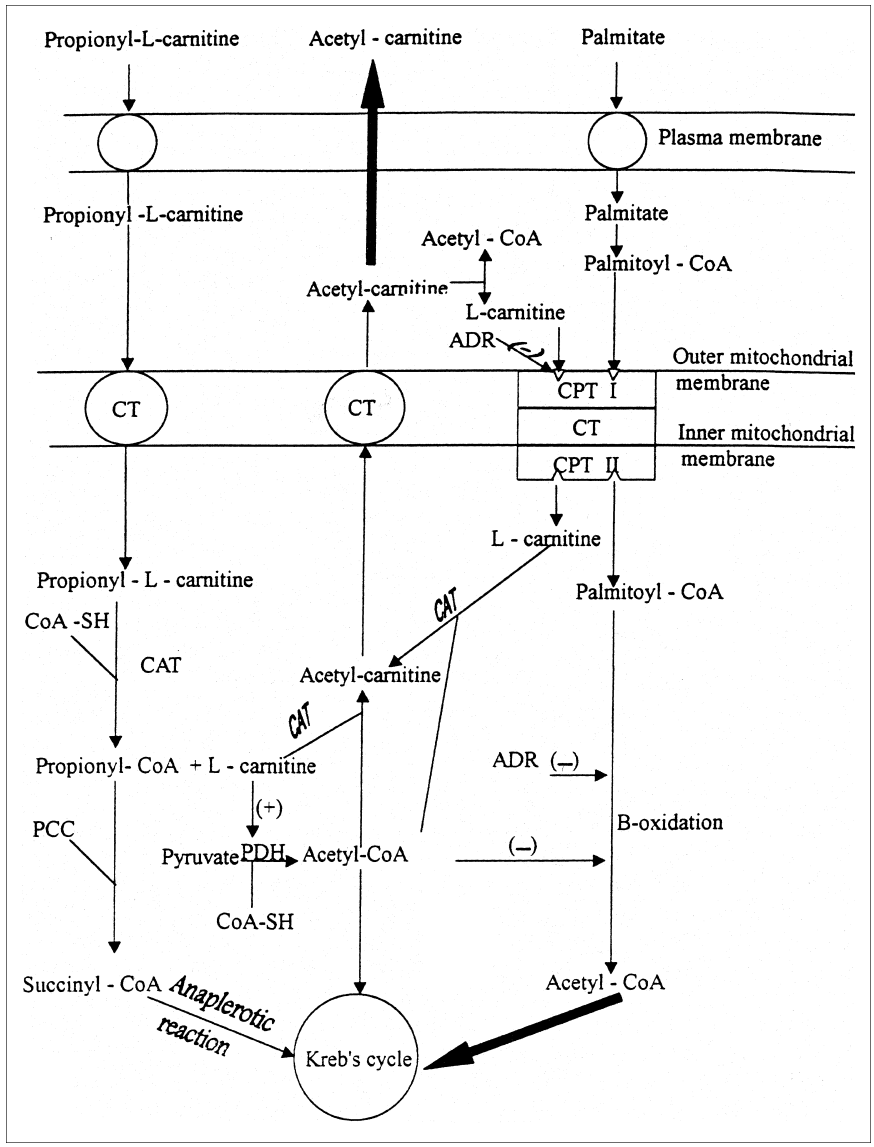

Fig. 4. Proposed protective mechanism by PLC against ADR-induced cardiomyopathy. CT, carnitine/acylcarnitine translocase; CAT, carnitine acetyl transferase; CPT I, outer carnitine palmitoyltransferase; CPT II, inner carnitine palmitoyltransferase; PCC, propionyl-CoA carboxylase; ADR, adriamycin; (−) and (+), indicate inhibition and stimulation, respectively.

oxidation of [U-^{14}C]glucose was significantly decreased by 18% in isolated cardiac myocytes. The decrease in glucose oxidation by PLC is not surprising since the levels of L-carnitine in cardiac myocytes play a role in the interaction between fatty acid and glucose oxidation. It has been reported that high levels of L-carnitine (5 mM) in cardiac myocytes increased $^{14}CO_2$ released from [1-^{14}C]palmitate and [1-^{14}C]pyruvate oxidation suggesting that L-carnitine increased both palmitate oxidation and pyruvate dehydrogenase (PDH) activity. This would seemingly violate the inverse relationship between fatty acid and glucose oxidation. However, acetyl-CoA generated by PDH in this model did not enter Kreb's cycle, but instead was converted to acetylcarnitine by CAT and transported out of the myocytes. Thus the actual oxidative metabolism of glucose decreased despite the increase in PDH activity. Acetyl-CoA generated from fatty acid oxidation was preferentially metabolized through the Kreb's cycle [22, 23] (Fig. 4).

Our study revealed that ADR inhibits palmitate oxidation in isolated myocytes (Fig. 1). These results are consistent with previous studies in both acute and chronic ADR cardiomyopathic models [6, 7, 26]. The inhibition of palmitate oxidation by ADR may be due to inhibition of one or more sites in the pathway of palmitate oxidation (Fig. 4) which includes: the transport of palmitate across the plasma membrane; its activation in the cytosol into palmitoyl-CoA by acyl-CoA synthetase; its transport across the inner mitochondrial membrane via the carnitine palmitoyltransferase system (CPT I, CT and CPT II); and finally its oxidation in the mitochondrial matrix through the β-oxidation cycle. Previous studies in our laboratory have suggested that ADR has no effect on either palmitate transport in isolated cardiac myocytes [22] or palmitate activation by acyl-CoA synthetase in isolated heart mitochondria [13].

The inhibition of palmitoyl-CoA oxidation (an index of CPT I) in isolated rat heart mitochondria by ADR (Table I and Fig. 2) suggests that CPT I may be the inhibition target of palmitate oxidation. This hypothesis is consistent with the data presented by Kashfi *et al.* [19] which demonstrated the inhibition of CPT I and CPT II by ADR in isolated heart and liver mitochondria.

The slight inhibition of CPT I by ADR in our study (Table II) is in good agreement with the data presented by Brady and Brady [27] which reported that CPT I is less sensitive to the inhibition by ADR than CPT II due to the lower cardiolipin in the outer mitochondrial membrane than the inner one. The interaction of ADR with cardiolipin, the structural phospholipid of the inner mitochondrial membrane, has previously been reported [28]. A recent study in our laboratory [13] reported that ADR may inhibit CPT I by depletion of its co-factor L-carnitine and/or ADR competes with L-carnitine for its binding site on CPT I. This is supported by the data demonstrating the reversal of ADR-induced inhibition of palmitate oxidation in myocytes (Fig. 1) and palmitoyl-CoA oxidation in isolated mitochondria by PLC (Fig. 2, Table I).

In this study the inhibition of palmitoyl-carnitine oxidation by ADR in isolated mitochondria is much more difficult to explain than that of palmitoyl-CoA (Fig. 3). Since palmitoyl-carnitine is a CPT I-independent β-oxidation substrate, therefore, its inhibition by ADR may be due to the decrease in its transport through the inner mitochondrial membrane via CT and/or the inhibition of its oxidation through the β-oxidation cycle which occurs in the mitochondrial matrix (Fig. 4). A previous study demonstrated the interaction of ADR with cardiolipin, the structural phospholipid of the inner mitochondrial membrane, forming ADR–cardiolipin complex [28]. This complex decreases the integrity of the inner mitochondrial membrane with the consequent decrease in the transport of palmitoyl-carnitine. If this hypothesis was correct, ADR should inhibit the oxidation of another CPT I-independent β-oxidation substrate such as medium and short-chain fatty acids. Previously, Sayed-Ahmed [22] and Abdel-aleem *et al.* [7] reported that ADR did inhibit the oxidation of both octanoate (medium-chain) and butyrate (short-chain) in isolated cardiac myocytes. It is of interest that two distinct systems of β-oxidation enzymes are present in mitochondria [29]. The first system is responsible for the oxidation of long-chain acyl-CoA esters and is bound to the inner mitochondrial membrane.

The second system is responsible for the oxidation of short- and medium-chain esters and is located in the mitochondrial matrix. Since it has been reported that ADR interacts with the inner mitochondrial membrane [28] to which long-chain β-oxidation enzymes are located, the inhibition of palmitoyl-carnitine oxidation by ADR could be due to the inhibition of a particular site in long-chain β-oxidation enzymes. On the other hand, the reversal of ADR-induced inhibition of palmitoyl-carnitine by PLC in mitochondria (Fig. 3) may be due to the reduction of the ADR–cardiolipin interaction by the L-carnitine portion of PLC, thus preserving the integrity of the inner mitochondrial membrane and probably protecting palmitoyl-carnitine transport. This speculation is consistent with previous studies which have reported the complete reversal of ADR-induced inhibition of octanoate and butyrate by L-carnitine (5 mM) in isolated cardiac myocytes [7, 22]. The interaction of L-carnitine with cardiolipin which is essential for expression of CT activity has been previously reported [30]. Another possible mechanism by which PLC could protect against ADR-induced inhibition of palmitoyl-carnitine oxidation is by reduction of the mitochondrial acetyl-CoA by L-carnitine and/or propionyl-CoA. This effect

stimulates β-oxidation by activating 3-ketoacyl-CoA thiolase which controls the final reaction in the β-oxidation cycle [31].

Under our experimental conditions, ADR showed a significant decrease in ATP concentration. These results are consistent with the data presented by Neri *et al.* [20]. The decrease in ATP by ADR may be due to inhibition of adenine nucleotide translocase and oxidative phosphorylation in mitochondria by fatty acid intermediates accumulated secondary to the inhibition of palmitate by ADR. Previously, Kobayashi and Fujisawa [32] reported that the accumulation of long-chain acyl-CoA in mitochondria inhibits adenine nucleotide translocase with a consequent decrease in the formation and utilization of ATP. A recent study in our laboratory [22] and another [23] have reported that the concentration of ADR (0.5 mM) that inhibited the oxidation of long-chain (palmitate), medium-chain (octanoate), and short-chain (butyrate) fatty acids in cardiac myocytes, has no effect on glucose oxidation. Therefore, the decrease in ATP production by ADR in our study could be due to the overall effects of ADR in the inhibition of fatty acid oxidation. On the other hand, the preaddition of PLC to ADR-incubated heart slices serves to restore ATP concentration by increasing the flux of acetyl-CoA through the Kreb's cycle and preventing the accumulation of toxic fatty acid intermediates, thus relieving the inhibition of adenine nucleotide translocase.

It is worth mentioning that glucose oxidation has no contribution in ATP recovery by PLC since it has been reported that this compound decreased glucose oxidation in isolated cardiac myocytes [25]. Therefore, the recovery of ATP by PLC is mainly due to the relieving of ADR-induced inhibition of palmitate oxidation. In conclusion, results of this study revealed that ADR induced its cardiotoxicity by inhibition of CPT I and β-oxidation of long-chain fatty acids with the consequent depletion of ATP in cardiac tissues, and that PLC can be used as a protective agent against ADR-induced cardiotoxicity.

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