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Doxorubicin induces early lipid peroxidation associated with changes in glucose transport in cultured cardiomyocytes

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Abstract

Doxorubicin (DOX) has not only chronic, but also acute toxic effects in the heart, ascribed to the generation of reactive oxygen species (ROS). Focusing on the DOX-induced early biochemical changes in rat cardiomyocytes, we demonstrated that lipid peroxidation is an early event, in fact conjugated diene production increased after 1-h DOX exposure, while cell damage, evaluated as lactate dehydrogenase (LDH) release, was observed only later, when at least one third of the cell antioxidant defences were consumed. Cell pre-treatment with α-tocopherol (TC) inhibited both conjugated diene production and LDH release. In cardiomyocytes, DOX treatment caused a maximal increase in glucose uptake at 1 h, demonstrating that glucose transport may represent an early target for DOX. At longer times, as the cell damage become significant, the glucose uptake stimulation diminished. Immunoblotting of glucose transporter isoform GLUT1 in membranes after 1-h DOX exposure revealed an increase in GLUT1 amount similar to the increase in transport activity; both effects were inhibited by αTC. Early lipid peroxidation evokes an adaptive response resulting in an increased glucose uptake, presumably to restore cellular energy. The regulation of nutrient transport mechanisms in cardiomyocytes may be considered an early event in the development of the cardiotoxic effects of the anthracycline.

Keywords: Lipid peroxidation; Doxorubicin; Cardiomyocyte; Glucose transport; Glucose transporter

1. Introduction

Doxorubicin (DOX), commercially and clinically known as adriamycin, is a highly effective antineoplastic drug against solid tumors and hematological malignancies and is widely used in both pediatric and adult patients [1]. However, its clinical use is often limited by undesirable side effects, especially a severe, cumulative and dose-dependent cardiotoxicity [2,3]. Besides chronic toxic effects, DOX has also acute toxic effects on the heart. The chronic, long-term effects, developed only after several weeks or months of treatment, include the onset of cardiomyopathy that often leads to congestive heart failure. The acute, short-term effects are clinically characterized by atrial and ventricular dysrhythmias, a perycarditis–myocarditis syndrome and acute hypertensive reactions occurring shortly after the administration of the drug [4].

Various mechanisms have been ascribed to the DOX long-term induced cardiotoxicity, including reduction of ventricular β-adrenergic receptor density [5], inhibition of nucleic acid and protein synthesis [6] as well as protease activities [7]. The mechanism of DOX-induced short-term toxicity is not fully elucidated, although acute alterations in platelet-activating factor, prostaglandins, histamine and intracellular calcium have been implicated [8]. One of the most accepted mechanisms for the anthracycline-induced early damage is now regarded as being mediated by a continuous generation of oxy radicals from DOX via a redox cycling. DOX undergoes redox activation in the presence of certain flavin enzymes and mitochondrial complexes I–III [9–12]. Recently, DOX was shown to undergo reductive activation at the reductase domain of the enzyme endothelial nitric oxide synthase [13] and at the level of the multienzymatic complex of fatty acid delta-6-desaturase [14] at a very early stage.

Conceivably, these reactive oxygen species (ROS) could then damage membranes and may profoundly affect membrane-associated proteins, including enzymes, receptors and

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transporters. A group of important membrane proteins is the specialized glucose transporters (for a review, see Ref. [15]). The heart expresses both the transporter isoform GLUT4, important for the normal function and properties of cardiac myocytes [16], and the GLUT1, which is thought to be at least in part responsible for the basal uptake of glucose [17]. The transporter isoform GLUT4, largely confined to an intracellular storage site, becomes recruited to the cell surface under the influence of several stimuli, such as insulin, contraction, and hypoxia [18–20]; also GLUT1 is recruited to the plasma membrane by several stimuli including insulin, metformin, serotonin and cathecolamies [21–23]. In many cell systems, it has been demonstrated that GLUT1 activity can be acutely stimulated also by oxidative stress [24,25]. The mechanism by which cells react to oxidate stress has been extensively studied but not completely understood. In M07, a human megakaryocytic cell line, the effect of H_2O_2 on transport V_{max} could be ascribed to a transporter translocation to the plasma membranes [26]. A stimulation of glucose transport by many stimuli through an activation of GLUTs preexisting in the plasma membrane has also been described [27]. Thus, we decided to verify whether DOX treatment, due to the ability of the drug to generate ROS, could be able to modulate glucose transport into the cardiac cell and possibly to influence the intracellular distribution of GLUT isoforms.

In this study, using primary cultures of neonatal rat ventricular cells, we have investigated the DOX-induced cascade of early biochemical changes in the heart, demonstrating the relationship between DOX-induced lipid peroxidation and acute alterations in glucose transport. The central role of early lipid peroxidation in the acute regulation of glucose transport into the cardiac cells was confirmed by means of α-tocopherol (αTC) pre-treatment.

2. Materials and methods

2.1. Materials

Doxorubicin hydrochloride, αTC, horse serum (HS), fetal calf serum (FCS), Ham F10 culture medium, phloretin, 2-deoxy-D-glucose (DOG), phenylmethylsulfonyl fluoride (PMSF), *N*-tosyl-L-lysine chloromethyl ketone (TLCK), *N*-tosyl-L-phenylalanine chloromethyl ketone (TPCK), 2,2′-azinobis (3-ethylbenzo-6-thiazoline-6-sulfonic acid) (ABTS) and Trolox were from Sigma Chemical (St. Louis, MO, USA). 2-Deoxy-D-[2,6-^{3}H]-glucose was from Amersham (UK); nitrocellulose paper (BA 83) was obtained from Schleicher and Schuell (Keene, NH, USA). Rabbit polyclonal antisera against rat GLUT1 and GLUT4, antirabbit IgG conjugated to horseradish peroxidase, and Western Blotting Luminol Reagent were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Sulfosuccinimidyl 6-(biotinamido) hexanoate (NHS-LC-biotin), streptavidin-agarose beads and Micro BCA protein assay reagent were purchased from Pierce (Rockford, IL, USA). UV-grade acetonitrile was from Prolabo (Paris, France). All the other chemicals and solvents were of the highest analytical grade.

Doxorubicin hydrochloride was dissolved in PBS at 1 mM concentration and stored at − 20 °C in the dark. The thawed solution was diluted with culture medium to the appropriate concentration and used immediately. αTC was dissolved in ethanol at 5 mM concentration, stored at − 20 °C, and diluted with culture medium to the appropriate concentration.

2.2. Cell cultures

Primary heart cell cultures were obtained by isolation of cardiomyocytes from the ventricles of 2–4 days old Wistar rats, as previously reported [28]. Cells were grown in 60 mm Petri dishes until confluence in nutrient mixture Ham F10 supplemented with 10% v/v FCS and 10% v/v HS. At confluence, cardiomyocytes were supplemented with 20 μM αTC. Twenty-four hours later, cultures were exposed to 1 μM DOX for different times. Appropriate control groups were processed in the same way in the absence of αTC or DOX.

2.3. Determination of conjugate dienes

The appearance of conjugate diene containing lipids was evaluated as an index of lipid peroxidation using the method of Burton et al. [29]. Cells were washed three times with control medium supplemented with 10% FCA and 10% HS, and two times with PBS. Preliminary experiments demonstrated that DOX is completely removed by these washes. All the cells were scraped off in ice-cold methanol, and extracted in chloroform/methanol/water (2:1:1 v/v). The chloroform layers from two extractions were combined and then dried under nitrogen. Samples were resuspended in a known volume of acetonitrile and the absorbance determined at 235 nm.

2.4. Determination of the total antioxidant activity (TAA)

TAA, a marker of the cell antioxidant status, was measured on the $800 \times g$ supernatant of cardiomyocytes scraped off in ice-cold buffer and homogenated as reported by Re et al. [30]. This method is based on the ability of the antioxidant molecules in the cell extract to reduce the radical cation of ABTS, determined by the decolorization of $ABTS^{\circ+}$ and measured as quenching of absorbance at 740 nm. Values obtained for each sample were compared to the concentration–response curve of a standard Trolox solution, and expressed as Trolox Equivalent Antioxidant Activity (TEAC, μmol/ml).

2.5. Lactate dehydrogenase (LDH) release

LDH release was monitored before cell scraping by collecting aliquots of medium from each dish; these aliquots

were then analyzed spectrophotometrically for LDH activity by measuring NADH levels at 340 nm [31].

2.6. Glucose transport assay

Glucose uptake was determined following Berridge and Tan [32], with slight modifications. Confluent cells on 60-mm culture plates were washed twice with ice-cold PBS followed by the addition of 1 ml of PBS at 37 °C. A mixture of 2-deoxy-D-[2,6-^{3}H]glucose (0.4 μCi/assay) and 1.0 mM unlabeled glucose analogue was added to each dish, and incubation performed for 5 min at 37 °C under conditions where the uptake was linear at least for 20 min. The uptake was stopped with phloretin (final concentration 0.2 mM). The cells were washed with PBS and scraped after the addition of 1 ml of lysis buffer (20 mM HEPES, 2 mM EDTA, 0.1% CHAPS and 0.1% Triton). Sample radioactivity was measured by liquid scintillation counting.

2.7. Biotinylation of plasma membranes

Biotinylation was performed as previously described [33]. Confluent cells were rinsed with ice-cold PBS at pH 8.0 followed by the addition of 1.5 ml of cold biotinylation buffer (120 mM NaCl, 30 mM $NaHCO_3$ and 5 mM KCl, pH 8.5) containing 0.1 mg/ml freshly added NHS-LC-biotin. After 30 min of gentle swirling at 4 °C, the medium was aspirated and the plates were washed with buffer containing 140 mM NaCl, 20 mM Tris and 5 mM KCl, pH 7.5. Cells were then scraped and pooled in 1 ml of hypotonic homogenization buffer containing 10 mM $NaHCO_3$ and a 100 μM concentration each of TPCK, TLCK and PMSF. After 10 min on ice, the cells were homogenized in a Potter homogenizer with 20 strokes, and 0.1 ml of buffer containing 1.5 M NaCl and 100 mM Tris (pH 7.0) was added. The homogenates were spun for 15 s at 18,000 × g to sediment nuclei. The resulting postnuclear supernatants were added to 1.5 ml Eppendorf Microfuge tubes containing 50 μl of streptavidin-agarose beads that had been sedimented following pre-equilibration with 1 ml of homogenization buffer. An additional 5 μl aliquot of 20 mM PMSF was added to each mixture. After gentle mixing of the samples at 4 °C for 30 min, the beads were pelleted and washed with 1 ml of homogenization buffer containing freshly added protease inhibitors. The final pellets were resuspended in Laemmli buffer and incubated at 65 °C for 30 min. The beads were once again briefly pelleted and the supernatants containing solubilized plasma membranes were removed and frozen overnight prior to use.

2.8. SDS-PAGE and Western blot analysis

Cell fractions containing equal amounts of protein (20 μg of protein/lane) were added with bromophenol blue and boiled for 3 min. Proteins were separated on 10% SDS-polyacrylamide gel using a Mini-Protean II apparatus (Bio-Rad Laboratories, CA, USA). Proteins were transferred electrophoretically to supported nitrocellulose membrane at 100 V for 60 min. Nonspecific binding was blocked by incubating in TBS/Tween, pH 8.0 containing 5% nonfat dried milk for 1 h at room temperature. Then, the nitrocellulose membranes were incubated overnight at 4 °C with rabbit polyclonal antiserum against rat GLUT1 or GLUT4 at 1:1000 dilution. Blots were washed with TBS/Tween and then incubated for 30 min at room temperature with anti-rabbit IgG conjugated to horseradish peroxidase diluted 1:2000 in TBS/Tween containing 5% nonfat dried milk. Membranes were washed with TBS/Tween and developed using Western Blotting Luminol Reagent. Semiquantitative analysis of specific immunolabeled bands was performed using a Fluor S image analyzer (BioRad Laboratories).

2.9. Protein determination

Protein concentration was determined using the Bradford method [34]. Before electrophoresis, because of the presence of high concentrations of SDS in some samples, the protein content was determined by means of a micro-BCA protein assay kit from Pierce, using bovine serum albumin as a standard.

2.10. Statistical analysis

Data are means ± S.D. of at least four different cell cultures. Statistical differences were evaluated using the Student's t-test.

3. Results

Peroxidation of unsaturated lipids leads to the formation of conjugate diene-containing lipids. The exposure of cardiomyocytes to DOX caused the formation of conjugate diene-containing lipids in the very early phases of treatment. In Fig. 1, conjugate diene production in control and αTC-supplemented cells is reported in function of the time of exposure to 1 μM DOX as 235 nm absorbance. αTC treatment did not influence conjugate diene production in cells not exposed to DOX independently of the time (data not shown). DOX caused a significant increase (about 1.6-fold) in conjugate diene levels at the first hour of treatment in comparison to cells not exposed to the drug, and a linear correlation between conjugate diene production and the time of exposure was revealed in the first three hours ($r = 0.996$, $P < 0.01$). A 6-h treatment did not evidence any significant difference in comparison to a 3-h treatment. Conjugated diene production in cells supplemented with αTC was similar to cells not exposed to DOX at each time point and significantly lower than in cells exposed to DOX and not supplemented with the antioxidant.

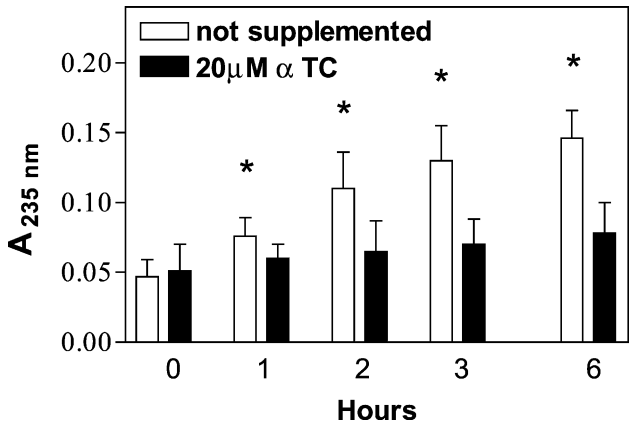

Fig. 1. Time course of the effect of 1 μM DOX on conjugated diene-containing lipids in cultured cardiomyocytes in the absence or presence of αTC. Conjugated diene production was measured as 235 nm absorbance as reported in Materials and methods. Data are means ± S.D. of five different cell cultures. Statistical analysis was performed by the Student's *t*-test comparing cardiomyocytes exposed to DOX vs. cardiomyocytes not exposed to DOX: $*P<0.001$. In comparison to the corresponding cells supplemented with 20 μM αTC, statistical analysis revealed a significant increase in conjugated diene production at each time of DOX treatment: $*P<0.001$.

In order to correlate lipid peroxidation to intracellular antioxidant status, TAA was evaluated in cardiomyocytes exposed to 1 μM DOX for different times (Table 1). TAA appeared always higher in cells not exposed than in cells exposed to DOX, and the decrease in the TAA of unsupplemented cells was significantly correlated to the time of exposure to the drug in the first three hours ($r=0.983$, $P<0.02$), in agreement with the observed production of conjugated dienes. A significant increase in TAA was achieved by supplementing cells with αTC, and the value remained always higher to that of unsupplemented cells at each time point.

To investigate the integrity of plasma membranes, LDH leakage from cultured cardiomyocytes, an indication of cell injury, was examined. The release of LDH from unsupplemented and αTC supplemented cardiomyocytes exposed to 1 μM DOX for different times is reported in Fig. 2. αTC pre-treatment did not influence LDH release in cells not exposed to DOX independently of the time (data not shown). An about 1.7-fold increase in LDH release was demonstrated in cardiomyocytes exposed to the anthracycline in the absence of αTC only after 3 h of DOX exposition. A 6-h treatment did not evidence any significant difference in comparison to the 3-h treatment. A protection was achieved by the addition of αTC to cardiomyocytes, LDH release value being similar to that observed in cells not exposed to the drug and significantly lower than in cells exposed to DOX and not supplemented with the antioxidant.

The time course of the effect of DOX treatment on DOG transport in cultured cardiomyocytes is reported in Fig. 3. DOG uptake is reported as the percentage of not exposed cells (25 787 cpm/mg protein/5 min = 100%). DOG uptake did not evidence significant differences at any time point in the absence of DOX treatment, indicating that the basal rate of glucose transport remained constant. Upon DOX exposure, DOG transport rose about 2-fold within 1 h, when compared to the initial, not exposed control cells. The treatment of cells for 2 h decreased the incremental increase in glucose transport due to 1 μM DOX exposure by 15%, although this value was not significantly different from that observed at 1-h treatment. The incremental increase in DOG transport was almost completely suppressed at 3- and 6-h

Table 1
Time course of the effect of 10^{-6} M DOX on cell TAA in cultured cardiomyocytes in the absence or presence of DOX

DOX treatment (h)	TEAC (μmol/ml)	
	Not supplemented	20 μM αTC
0	96.17 ± 3.19	159.86 ± 5.11
1	80.16 ± 3.18*	140.17 ± 4.18*
2	70.45 ± 4.53*	128.84 ± 5.36*
3	63.33 ± 4.13*	125.92 ± 4.25*
6	61.43 ± 3.89*	124.89 ± 5.12*

Total antioxidant activity was evaluated by measuring the ability of the cell extract to reduce the radical cation of ABTS, as reported in Materials and methods. Values obtained for each sample were compared to the concentration–response curve of a standard Trolox solution, and expressed as TEAC (μmol/ml). Data are means ± S.D. of four different determinations. Statistical analysis was performed by the Student's *t*-test comparing cardiomyocytes exposed to DOX vs. the corresponding cardiomyocytes not exposed to DOX: $*P<0.001$. In comparison to the corresponding unsupplemented cells, statistical analysis revealed a significant increase in TEAC at each time of DOX treatment: $P<0.001$.

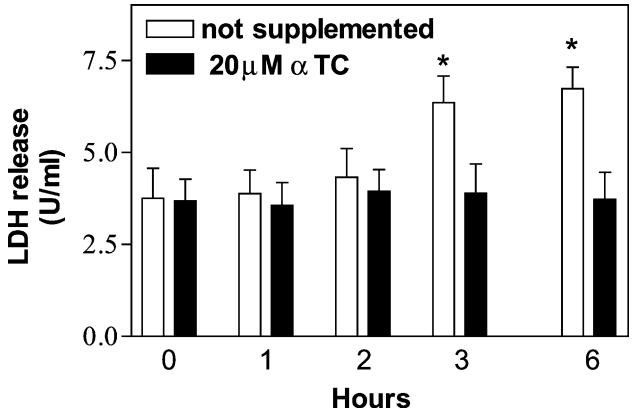

Fig. 2. Time course of the effect of 1 μM DOX on LDH release in the culture medium of cardiomyocytes in the absence or presence of αTC. LDH release was measured on aliquots of culture media as reported in Materials and methods. Data are means ± S.D. of five different cell cultures. Statistical analysis was performed by the Student's *t*-test comparing cardiomyocytes exposed to DOX vs. cardiomyocytes not exposed to DOX: $*P<0.001$. In comparison to the corresponding cells supplemented with 20 μM αTC, statistical analysis revealed a significant increase in LDH release only at 3 and 6 h of DOX treatment: $*P<0.001$.

DOX treatment. Pre-treatment of cardiomyocytes with αTC for 24 h completely abolished the DOX-induced enhancement in DOG uptake, but did not influence the basal glucose transport.

In order to clarify the molecular basis for the early stimulation of glucose transport by DOX, immunoblotting analysis was performed in a plasma membrane-enriched fraction of cardiomyocytes exposed to DOX for 1 h, a condition at which we observed the maximal stimulation of glucose uptake without causing LDH leakage. In order to quantify the relative abundance of GLUTs at the surface of cardiomyocytes previously exposed to DOX, in the absence and in the presence of αTC, Western blot analysis of plasma membrane-enriched fraction with anti-GLUT4 antibodies was performed and GLUT4 relative abundance was quantified using scanning densitometry; no significant differences in GLUT4 amount in membranes of cells exposed to the drug was detected in comparison to cells not exposed (data not shown). Similarly, Western blot analysis of plasma membrane-enriched fraction with anti-GLUT1 antibodies was performed, and GLUT1 relative abundance was quantified using scanning densitometry. Fig. 4A shows a representative immunoblot evidencing the effect of 1-h DOX treatment on GLUT1 content in the plasma membrane-enriched fraction, in the absence and presence of αTC. The intensity of GLUT1 immuno-reactive protein in the plasma membrane fraction of DOX-treated cells was increased compared to not exposed cells. Densitometric analysis indicate that GLUT1 content in the plasma membranes of cells treated with DOX was increased by about 2.0-fold as compared to GLUT1 from untreated cells (Fig. 4B). αTC supplementation was able to completely suppress the recruitment of the transporter to the membrane fraction without influencing basal GLUT1 level. Interestingly, the DOX-dependent increase in glucose transport, following 1-h treatment, is of similar extent to the increase in GLUT1 content in the plasma membrane-enriched fraction.

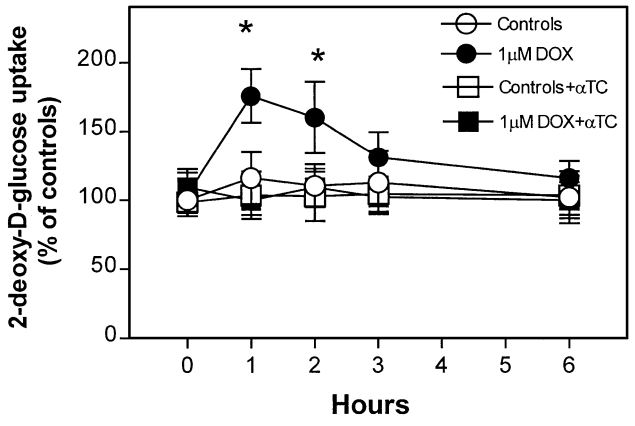

Fig. 3. Time course of the effect of 1 μM DOX on DOG uptake in cultured cardiomyocytes in the absence or presence of αTC. Confluent cardiomyocytes were exposed to 1 μM DOX for the indicated periods of time in the absence or the presence of 20 μM αTC. At the end of the incubation periods, the plates were rinsed and DOG transport was measured for 5 min as described in Materials and methods. Data are reported as percent of control cells (no DOX and no αTC; 25 787 cpm/mg protein/5 min = 100%). Data are means ± S.D. of four different cell cultures. Statistical analysis was performed by the Student's *t*-test comparing cardiomyocytes exposed to DOX vs. cardiomyocytes not exposed to DOX: $*P < 0.001$. In comparison to the corresponding cells supplemented with 20 μm αTC, statistical analysis revealed a significant increase in glucose uptake only at 1 and 2 h of DOX treatment: $*P < 0.001$.

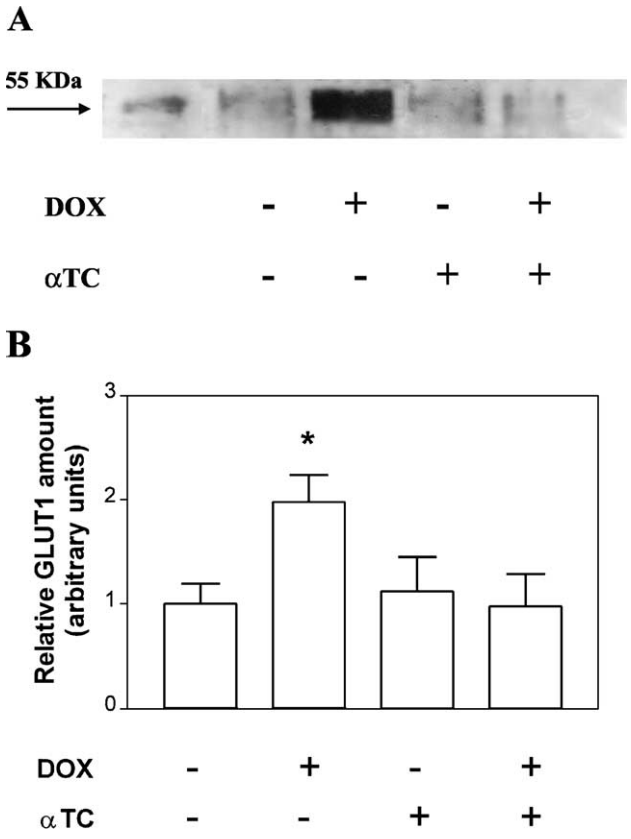

Fig. 4. Western blot and densitometric analysis of GLUT1 content in plasma membrane-enriched fraction of cultured cardiomyocytes after 1 h exposure to 1 μM DOX in the absence or presence of αTC. Plasma membrane-enriched fraction was prepared from cultured cardiomyocytes after 1 h exposure to 1 μM DOX as described in Materials and methods. (A) Representative immunoblot showing the effect of 20 μM αTC on GLUT1 translocation in response to 1 h exposure to 1 μM DOX. Twenty micrograms of protein per lane was separated by SDS-PAGE on 10% polyacrylamide gels, transferred to nitrocellulose membrane and immunoassayed using anti-GLUT1 antibodies. Corresponding bands migrating at ≈ 55 kDa are shown. (B) Results of scanning densitometry analysis performed on four independent autoradiographs for GLUT1 are presented. Relative amounts (means ± S.D.) are in arbitrary units and compared to the corresponding GLUTs from cells not exposed to the drug; $*P < 0.001$ vs. controls.

4. Discussion

DOX-induced cardiotoxicity occurs in a biphasic manner, an acute phase and a delayed phase, characterized by electrophysiological abnormalities and congestive heart failure,

respectively [35]. One hypothesis for the mechanism for the acute phase is the involvement of free radicals [9–14] that could then damage membrane phospholipids leading to lipid peroxidation. The cultured cardiomyocyte model has enabled us to demonstrate that lipid peroxidation is an early event. Similar results were obtained by Luo et al. [36] who demonstrated that aldehydic lipid peroxidation products in rat heart tissue increased significantly after 1-h drug treatment. DOX-induced lipid peroxidation could potentially account for a parallel increase in LDH release. LDH leakage was significantly increased only after 3-h treatment, indicating that lipid peroxidation is a very early event that induces cell damage only at longer times. In rats treated with a bolus injection of DOX, creatine kinase activity in rat plasma, a clinical marker for the determination of cardiac damage, was significantly elevated only 2–4 h after treatment [36] and in rats receiving DOX for two or three doses every third day, a significant creatine kinase release was detected only at day 7 [37].

Differences in the antioxidant capacity of tissues can explain why cardiac tissue develops early lipid peroxidation after DOX administration, whereas other tissues, such as the liver, are relatively resistant to damage. It has been reported that the activity of antioxidant enzymes is lower and the susceptibility to oxidative stress is higher in rat heart than in other tissues such as the liver [38,39]. In fact, TAA was greatly affected by DOX treatment and it decreased almost linearly as the time of exposure to the drug increased, but cell damage was evidenced only when at least one-third of the cell antioxidant defences were consumed and free radicals exceed the capacity of the cellular intrinsic free radical scavenging system, becoming cytotoxic.

The relationship between DOX-induced lipid peroxidation and LDH release was demonstrated by cell pre-treatment with αTC, in which both phenomena were significantly inhibited by the antioxidant. Vitamin E supplementation significantly increased the TAA in comparison to unsupplemented cells, and the TAA value remained always higher than that observed in not treated, not supplemented cells. The 20 μM αTC dose used in this study has been reported to increase cellular αTC content and to reduce membrane lipid alteration, to enhance the recovery of contractile function and to reduce the accumulation of calcium in isolated rat hearts exposed to global ischemia and reperfusion [40]. Recently, we demonstrated that such a αTC dose was able to inhibit both lipid peroxidation and alterations in fatty acid composition induced by a longer (24 h) DOX treatment in cultured cardiomyocytes [41]. Moreover, while cardiomyocytes not exposed to DOX contracted spontaneously in culture with steady, deep, rhythmic contractions at rates of 150 ± 32 beats per minute, chronic exposure to DOX for 24 h caused an asynchrony in the contractile activity of exposed cultures leading to a slowing of beating rate to 86 ± 27 beats per minute. Cardiomyocytes pretreated with αTC exhibited a maintenance of contractile function with a beating rate of 146 ± 29 beats per minute (S. Hrelia et al., unpublished data).

DOX has been reported to cause inhibition of β-oxidation of long-chain fatty acids [42] with consequent depletion of cardiac ATP and switch to anaerobic glycolysis [43]. Since in the heart glucose transport limits the glycolytic rate, its regulation may be important in maintaining the cell energy status. The present study provides a first direct demonstration that the glucose transport system in the cardiomyocytes may represent one of the early targets of the anthracycline. DOX caused the maximal increase in DOG uptake at 1-h treatment, when no detectable cell damage was evidenced. Only at longer times of drug exposure the incremental increase in DOG uptake diminished, as the progressive, irreversible cell damage became significant. The relationship between the increase in glucose transport and the increase in lipid peroxidation was evidenced by the ability of vitamin E to counteract this effect.

Glucose transport may be determined by the number of glucose transporters in the plasma membrane, and/or by the intrinsic activity of these transporters. Cardiac myocytes express both GLUT4 and GLUT1, and although GLUT4 is quantitatively the most important glucose transporter expressed in these cells, GLUT1 is also expressed at a substantial level. GLUT4 levels in membrane-enriched fractions did not change following DOX treatment; similar results were obtained by Rudich et al. [44] who demonstrated the impaired insulin-stimulated GLUT4 translocation following oxidative stress in 3T3-L1 adipocytes. Western blot and densitometric analysis of GLUT1 in cardiomyocyte membrane-enriched fractions after 1-h exposure to 1 μM DOX revealed that GLUT1 amount had approximately a 2-fold increase. Although the accuracy of the transport assay was higher in comparison to the densitometric analysis, the increase in GLUT1 transporter isoform content correlates well to the degree of enhancement in glucose transport and, similarly, αTC pre-treatment was able to inhibit the enhancement both in glucose transport and in GLUT1 content. Since glucose transport is largely determined by the number of glucose transporters present in the plasma membrane of cardiomyocytes, the effect of DOX on early regulation of glucose transport is likely to be based on the observed changes in GLUT1 distribution. Enhanced glucose uptake in cardiac cells can be achieved by rapid translocation of preexisting transporters from intracellular pools to the plasma membrane. In the case of short-term DOX exposure, glucose transport stimulation is an early phase response involving redistribution of preexisting GLUT1 transporters and different to that observed following anoxia, glucose deprivation or treatment with iron chelators, which occurs only later and involves GLUT1 gene induction [45,46]. The role of ROS produced by DOX in mediating GLUT1 recruitment to the plasma membrane is demonstrated by the effect that αTC had in preventing glucose uptake stimulation and transporter translocation. Interestingly, the effect of DOX-induced early lipid peroxidation on glucose transporter redistribution and expression in plasma membrane seems to be a unique property of the heart. In fact, it has recently

been demonstrated that DOX treatment did not change the amount and the appearance of GLUT1 transporter in cancer cells, as Erlich ascites carcinoma cells [47].

5. Conclusions

The present work focuses on the events that occur prior to irreversible cell damage caused by DOX to cardiomyocytes and demonstrates that DOX-induced lipid peroxidation is the main culprit of the short-term damage that set the stage of an adaptive response which resulted in a increased glucose uptake, presumably to restore cellular energy. Although the molecular mechanism by which DOX triggers redistribution of GLUT1 and thus regulates glucose uptake in heart cells remains to be elucidated, data here reported suggest that regulation of nutrient transport mechanisms in cardiomyocytes may be considered a peroxidation-dependent early event in the development of the cardiotoxic effects of the anthracycline, and set the stage of future interventions in counteracting the side effects of the clinical use of DOX.

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