Disorders Ca^{2+} Homeostasis in Rat Cardiac Mitochondria Affected by Adriamycin

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Summary: To determine the mechanism of adriamycin (ADR) induced cardiotoxicity, the effects of ADR on the Ca^{2+} uptake and release of cardiac mitochondria were investigated. Male Wistar rats were injected intraperitoneally with 4 mg/kg body weight of ADR for 6 consecutive days, and mitochondria were isolated from heart muscle. Ca^{2+} uptake and release of cardiac mitochondria were measured spectrophotometrically using a metallochromic dye antipyrilazo III. The total calcium content was determined by atomic absorption. ADR administration resulted in reductions of the Ca^{2+} uptake velocity, Na^+ - Ca^{2+} exchange velocity and the calcium content in cardiac mitochondria. The data reported here is useful for understanding the pharmacological effect of adriamycin to unfavourable side-effect, cardiotoxicity.

Key words: adriamycin—cardiotoxicity—mitochondria— Ca^{2+} -uptake— Na^+ - Ca^{2+} exchange

Introduction

Adriamycin (ADR) is one of the most effective antitumor agents. However, the clinical applications of ADR have been limited because of its unfavourable side effects. There are many reports concerning the causes of its cardiotoxicity, but the precise mechanisms are still unknown (Doroshov et al. 1980). Thayer (Thayer, 1984) suggested relationship between the elevation of serum lipid peroxides and the development of ADR-induced cardiomyopathy. And recent studies show that cardiac mitochondria are responsible for formation of semiquinone free radical, active oxygen and consequent radical-initiated lipid peroxidation (Davies et al. 1983; Nohl and Jordan, 1983; Pollakis et al. 1984). We have already reported the following biochemical changes in cardiac mitochondria

affected by ADR: elevation of lipid peroxide (Ogura, 1982; Yoon et al. 1983), decrease of CoQ_{10} content (Ogura, 1982), uncoupling of phosphorylation (Ogura, 1982; Yoon et al. 1983) and decline of riboflavin content (Hino et al. 1985).

In general, the intracellular distribution of Ca^{2+} is known to play extremely important role in the regulation of cellular viabilities. Disorders of Ca^{2+} homeostasis of cardiac cells have been reported to associate with several types of cardiomyopathy (Farber, 1981; Nysten and Wrogemann, 1983; Jasmin and Proschek, 1984). Disruption of Ca^{2+} homeostasis of cardiac mitochondria may be one of the mechanisms of ADR-induced cardiomyopathy. In this paper, we report the effect of ADR on Ca^{2+} uptake velocity, Na^+ - Ca^{2+} exchange velocity and calcium content in cardiac mitochondria.

Materials and Methods

Male Wistar rats weighing about 200 g were injected intraperitoneally with 4 mg/kg of ADR (adriacin, Kyowa Hakko Kogyo Co. Ltd.) for 6 consecutive days (Ogura, 1982). On the 7th day from the original injection, the animals were sacrificed by decapitation. The heart was excised quickly and placed in an ice-cold solution of 70 mM sucrose, 220 mM mannitol and 5 mM morpholinopropane sulfonic acid. Mitochondria were prepared from heart muscle according to the procedure of Ogura et al. (Ogura et al. 1986). The protein concentration of the mitochondrial suspension was determined by the biuret method with bovine serum albumin as a standard (Gornall et al. 1949).

1) Ca^{2+} uptake and Na^{+} -induced Ca^{2+} release

Ca^{2+} uptake and Na^{+} -induced Ca^{2+} release were measured by the method of Wolkowicz et al. (Wolkowicz et al. 1983). The reaction medium consisted of 70 mM KCl, 0.225 M sucrose, 9 mM HEPES, 1.5 mM KH_2PO_4 , pH 7.3, 80 μM antipyrilazo III, 5 mM potassium succinate, 10 μg rotenone with 3 mg protein in a final volume of 3 ml at 24°C. Following a 1–2 min incubation period at 24°C, the Ca^{2+} uptake was initiated by the addition of 10 μl of 4 mM CaCl_2 (40 n moles). The Ca^{2+} uptake by mitochondria was monitored by dual wavelength spectroscopy using antipyrilazo III at the wavelength pair 720–790 nm by means of Hitachi 557 dual wavelength spectrophotometer. After Ca^{2+} uptake and prior to the Na^{+} addition, 1 μM ruthenium red was added to inhibit further Ca^{2+} uptake. The Na^{+} -induced Ca^{2+} release was initiated by 3 mM of sodium chloride (Na^{+}). Five n moles of Ca^{2+} was added to the medium for calibration.

2) Free Ca^{2+} content in the mitochondria

Mitochondria (2 mg of protein) were incubated in the medium for 1 min at 24°C

in the same manner as indicated in the Ca^{2+} uptake experiment. Ruthenium red (1 μM) was added to inhibit energy-dependent Ca^{2+} uptake, and the subsequent Ca^{2+} release was determined following addition of the Ca^{2+} ionophore A23187 (10^{-7}M).

3) Total calcium content in mitochondria

Total calcium content in mitochondria were measured by the method of (Coll et al. 1982). Two tenths ml of the concentrated mitochondrial suspension containing approximately 20 mg of protein/ml was acidified with 0.4 ml of 14% (W/V) perchloric acid. The suspension was sonicated for 5 sec and centrifuged for 30 sec in an Eppendorf centrifuge. An aliquot of the supernatant (0.3 ml) was diluted to 3 ml with a mixture of 0.3 ml of strontium chloride (1000 ppm) and 0.3 ml of potassium chloride (1000 ppm) and 2.1 ml of distilled water. The mitochondrial calcium content was determined by atomic absorption spectroscopy with Hitachi 180-80 Atomic Absorption Spectrophotometer.

Results

Figure 1 indicates the Ca^{2+} uptake and Na^{+} -induced Ca^{2+} release of cardiac mitochondria in the control (A) and ADR treated (B) animals, as monitored by antipyrilazo III. The Ca^{2+} uptake velocity and release velocity are shown in Table 1. The Ca^{2+} uptake and release velocities were expressed as nmoles of Ca^{2+} /mg protein/min. Both the Ca^{2+} uptake velocity and the Na^{+} -induced Ca^{2+} release velocity decreased in the ADR treated group. To determine the influence of ADR on the net amount of calcium content in mitochondria, two kinds of methods were applied to detect the mode of calcium located in the matrix: A23187 and atomic absorption determinations. The experiment shown in Fig. 2 indicates the net Ca^{2+} release moni-

Fig. 1. The uptake and release of Ca^{2+} by rat heart mitochondria, as measured with the indicator antipyrylazo III. CaCl_2 was added at the point indicated to give 40 n moles. At the completion of the uptake phase, $1\text{ }\mu\text{M}$ of ruthenium red was added to inhibit further uptake and the release of Ca^{2+} was initiated by the addition of 10 mM of NaCl. A: untreated B: ADR treated.

TABLE 1

The effect of adriamycin on the Ca^{2+} uptake and Na^+ -induced Ca^{2+} release velocity of cardiac mitochondria

Treatment	Ca^{2+} uptake velocity	Ca^{2+} release velocity
control	106 ± 21	11.0 ± 1.9
ADR	$45 \pm 27^{**}$	$8.3 \pm 2.5^*$

n moles/mg protein/min

$^{**} p < 0.01 (n=6)$ $^* p < 0.05 (n=7)$

TABLE 2

The effect of adriamycin on the calcium content in cardiac mitochondria

Treatment	free Ca^{2+} (A23187)	Total calcium (A.A.)
control	11.0 ± 4.1	11.2 ± 2.8
ADR	$7.1 \pm 1.6^*$	$7.8 \pm 1.0^*$

n moles/mg protein

$^* p < 0.05 (n=7)$

Fig. 2. Quantitation of A23187 releasable calcium from rat cardiac mitochondria. Mitochondria (2 mg protein) were incubated at 24°C in a reaction medium, containing $80\text{ }\mu\text{M}$ antipyrylazo III. The concentrations of additions were: ruthenium red (RR), $1\text{ }\mu\text{M}$; A23187, $0.1\text{ }\mu\text{M}$.

tored by antipyrilazo III after addition of 10^{-7} M of A-23187 in the presence of 1μ M ruthenium red. Table 2 shows the Ca^{2+} released from cardiac mitochondria by treatment with Ca^{2+} ionophore A-23187. In Table 2, the total calcium content determined by atomic absorption spectroscopy is also shown. The total calcium and free Ca^{2+} contents were lower in the ADR treated group, as compared with those of controls ($n=7$, $p<0.05$). It was of particular interest to note that the free Ca^{2+} released in the presence of A-23187 was almost the same as the extractable calcium measured by atomic absorption method.

Discussion

The intracellular distribution of Ca^{2+} , particularly the cytosolic free Ca^{2+} concentration, is important in regulation of cardiac cellular activities. Fiskum and Lehninger (Fiskum and Lehninger, 1982) suggested that the Ca^{2+} transport processes of mitochondria work together with the endoplasmic reticulum to maintain the cytosolic free Ca^{2+} concentration. Many biochemical and pharmacological studies have emphasized that the mitochondrial Ca^{2+} transport system plays a principal role in the regulation of intramitochondrial Ca^{2+} content (Williamson et al. 1981; McCormack and Denton, 1984; Robertson et al. 1984). Mitochondrial Ca^{2+} homeostasis is considered to play an important role in the regulation of myocardial cellular processes. In the present paper, the homeostasis associated with Ca^{2+} uptake and release from heart mitochondria was studied in ADR-treated animals. The Ca^{2+} uptake velocity in ADR-treated rats was found to be significantly lower than that of control. Energy of Ca^{2+} uptake is derived from membrane potential generated by electron transport system (Lehninger et al. 1967). Sodium-induced Ca^{2+} efflux velocity was significantly lower in the ADR-treated mito-

chondria. The Na^{+} -induced Ca^{2+} release is known to occur as the result of electro neutral exchange to sodium (Affolter and Carafoli, 1980). The exchange velocity has been found to be influenced by the energy state of the inner membrane and the calcium content in matrix (Goldstone et al, 1983). Both the amount of ionized Ca^{2+} and the total calcium content in ADR-treated animals were significantly lower than those of the controls. Also, the total calcium content was close to that of ionized calcium in both normal and ADR-treated animals. All the extractable calcium by treatment with perchloric acid located in cardiac mitochondria may be able to be released as ionized calcium.

The detailed mechanism of ADR disturbance on the Ca^{2+} transport of cardiac mitochondria has not been clarified yet. However, it has been suggested that the physical structure of mitochondrial membrane may play a significant role in the regulation of Ca^{2+} concentration and transportation (Fiskum and Lehninger, 1982). In our separate paper (Sugiyama et al. 1986), the physical states of cell membrane treated with ADR was determined by the method of ESR spin labelling technique. Results obtained with ESR showed the decreased of membrane fluidity in ADR-treated group. And also we found the linear correlation between the membrane fluidity and the respiratory control ratio on the mitochondria (Shin, 1987). Therefore the alteration of the membrane physical properties causes deleterious effects on Ca^{2+} transport system of cardiac mitochondria.

Recent studies have indicated that the cytotoxic effect of ADR is related to the formation of lipid peroxide following semiquinone free radical formation (Ogura, 1982; Davies et al. 1983; Thayer, 1984). There are several papers suggesting the relationship between membrane lipid peroxidation and change of Ca^{2+} homeostasis (Bellomo et al. 1982; Moore et al. 1983).

The disorders of Ca^{2+} homeostasis of cardiac mitochondria may have a close association with degeneration of membrane affected by lipid peroxidation. These experiments may help to provide basic information for understanding the pathogenesis of cardiotoxicity induced by ADR.

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