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Effect of Dietary Coenzyme Q_{10} on Lipid Peroxidation in Adriamycin-Treated Rats

— III. Effect on Myocardial Ultrastructural Changes —

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= ABSTRACT =

The present study was designed to evaluate the effect of pretreatment with coenzyme Q_{10} on adriamycin-induced myocardial ultrastructural changes in rats. Except control group, 6 treatments included three levels of dietary coenzyme Q_{10}(0, 0.1 or 0.5g/kg diet) and two levels of ADR(1.0 or 2.0mg/kg B.W/week). Adriamycin treatment significantly decreased growth performance of rats. But this decrement was not modified by dietary supplementation of coenzyme Q_{10}. Electron microscopic examination revealed a progression of myocardial lesions were dependent upon the level of ADR injection. The most frequently observed fine structural alterations in rat myocardium were mitochondrial swelling, dilation of the sarcoplasmic reticulum and the appearance of a perinuclear vacuolization. But these structural changes were somewhat reduced by dietary supplementation of coenzyme Q_{10}.

KEY WORDS : coenzyme Q_{10} · adriamycin · myocardial ultrastructure.

Introduction

Adriamycin(ADR) is an antitumor antibiotic of anthracycline group with a broad spectrum of therapeutic activity[1], but the use of ADR in chemotherapy is limited by its cardiotoxicity[2]. Although the precise mechanism of ADR-induced cardiotoxicity is unexplained, it is suggested that one probable pathway may be the induction of peroxidation in cardiac lipids[3]. It is also considered that the ultrastructural alteration of myocardial cell may represent an important aspect of ADR toxicity. Revis and Marusic[4] have suggested that the functional consequences of cardiac toxicity by ADR, namely, alterations in the control of both myocardial calcium transport and the mitochondrial electron transport chain are a reflection of the histologic feature of this cardiomyopathy.

This cardiotoxicity of ADR were found to be reduced by pretreatment of the animals with the free radical scavenger, tocopherol[5][6]. In addition, the combined use of coenzyme Q_{10} with ADR has been recommended for reduction of the cardiotoxicity that occurs during cancer chemotherapy[7]. Coenzyme Q is essential to life, but the coenzyme Q_{10}

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required by the human body can be produced from other coenzyme Q_s in the diet. However, the body does not have the ability to synthesize it from individual chemical building blocks that are bonded together to form coenzyme Q[8]. From this perspective the original sources of coenzyme Q are only available through nutrition, even though it might not be considered a true vitamin. Takeshige et al[9] have demonstrated that reduced form of coenzyme Q_{10} functions as a potent antioxidant against membrane lipid peroxidation in submitochondrial particles. Ultrastructurally, coenzyme Q_{10} has been shown to prevent mitochondrial deformity during episodes of ischemia[10]. The present study, therefore, was undertaken to examine the effect of pretreatment with coenzyme Q_{10} on myocardial ultrastructural changes in ADR-treated rats.

Materials and Methods

1. Experimental animal and care

Animals used were male rats of Sprague-Dawley strain. Weanling rats were fed a basal diet for 4 weeks until they reached about 250g body weight. Thereafter, they were assigned to 7 experimental groups of 10 rats on the basis of their body weight and were individually housed in hanging stainless steel cages with wire-mesh bottoms. Rats were fed the experimental diets with ADR administration for 4 weeks after feeding experimental diets without administration of ADR for 4 weeks. Experimental design was described in Table 1. Experimental animal and diet were the same as in the previous paper [11]. Namely, two experimental diets(A1Q1, A2Q1 group) consisted of basal diet containing 0.1g coenzyme Q_{10} per kg of diet. Other two experimental diets(A1Q2, A2Q2 group) contained 0.5g coenzyme Q_{10} per kg of diet and the others(control, A1Q0, A2Q0 group) contained the basal diet without coenzyme Q_{10}. Coenzyme Q_{10} was supplied by Eisai pharmaceutical Company Ltd.(Tokyo, Japan). Except control rats, a dose of 1(A1Q0, A1Q1, A1Q2) or 2 mg ADR(A2Q0, A2Q1, A2Q2)/kg of body weight was injected to these animals intraperitoneally on the same day every week. Body weight and feed consumption were recorded weekly. Feed efficiency ratio(FER) was calculated by dividing body weight gain with feed intake.

Table 1. Experimental design

Group	Diet composition	Treatment
C	Basal diet	Saline[3]
A1Q0	Basal diet	ADR 1[4]
A1Q1	Basal diet+Co Q_{10} 1[1]	ADR 1
A1Q2	Basal diet+Co Q_{10} 2[2]	ADR 1
A2Q0	Basal diet	ADR 2[5]
A2Q1	Basal diet+Co Q_{10} 1	ADR 2
A2Q2	Basal diet+Co Q_{10} 2	ADR 2

[1] 0.1g of coenzyme Q_{10}/kg of diet
[2] 0.5g of coenzyme Q_{10}/kg of diet
[3] 1.0mg of saline/kg of body weight/week
[4] 1.0mg of ADR in saline/kg of body weight/week
[5] 2.0mg of ADR in saline/kg of body weight/week

2. Electron microscopic studies

1) Morphological study

Tissue was removed from the left lateral lobe of the heart while the animal was under ether anesthesia. Tissue samples were minced into small cubes, fixed in 1% osmium tetraoxide with caccodylate buffer for 1 hour at 4℃ and for 30 minutes at room temperature. And then the tissue was dehydrated in a graded ethanol series and was embedded in Epon 812. The blocked samples were out on a Porter-Blum MT-2 ultramicrotome with glass knives and then were picked up on uncoated gribs and stained with lead hydroxide. Finally, the sections were examined in a Hitach H-600 electron microscope.

2) Morphometric study

The morphometric sampling procedure was ba-

sed on the technique of Weibel at al[12]. For electron microscopic morphometry, from each group 30(5 rats×6) electron micrographs were made at a magnification of 40,000 X. Each density estimation of mitochondria was applied to point-counting method using multipurpose test grid(84 line/90×90.9 mm). The number of points counted by point-counting method was applied to the formula given by Weibel et al[13].

3. Statistical analyses

In the statisical analysis, the treatment effects were followed by one-way analysis of variance and Duncan's new multiple range test[14].

Results and Discussion

1. Growth performance

As shown in Table 2, there were significant differences in body weight gain between control and six ADR-treated groups. Adriamycin-treated rats were significantly decreased in the body weight gain compared with control rats. But the difference between rats injected lower and higher dose of ADR(A 1Q0 vs A2Q0) was less profound than that in the simultaneous use of ADR and coenzyme Q_{10} such as experiment 1 of the previous paper[11]. Body weight gain of groups treated higher dose of ADR was significantly smaller in comparison with that of A_1Q_2 group. However, there was no significant difference in body weight gain between A_2Q_2 group and A_1 groups. These findings indicated that pretreatment with dietary coenzyme Q_{10}, even if a little, has an effect on weight gain of rats.

Feed intake greatly depressed in ADR-treated rats, especially at higher level. But these decreases were reduced by dietary supplementation of coenzyme Q_{10}. Feed efficiency ratio(FER) was decreased with increasing level of ADR treatment, but the effect of dietary coenzyme Q_{10} on FER within the same ADR levels was negligible.

Present results revealed that ADR treatment had influence on growth performance of rats. Rats injected higher level of ADR had less weight gain compared to control rats. On the other hand, dietary supplementation of coenzyme Q_{10} affected slightly the growth rate of rats. According to the report by Olson and Capen[15], ADR-treated rats developed initial weight loss followed by gradual increment of body weight. Kim et al[16] and Mettler et al[17] also reported a similar relationship between ADR treatment and growth performance. But Ishikawa et al[18] presented that coenzyme Q_{10} treatment did not change the body weights of patients with congestive heart failure. These results were partially consistent with those of present study.

Table 2. Effect of dietary coenzyme Q_{10} on growth performance of ADR treated rat

Group	Initial body wt. (g)	Final body wt. (g)	Weight gain (g/day)	Feed intake (g/day)	FER
C	246.4± 15.8	455.6± 37.4^{a}	3.17± 0.45^{a}	22.30± 1.45^{a}	0.142± 0.017^{a}
A1Q0	246.4± 15.3	409.1± 20.9^{b}	2.46± 0.23^{bd}	20.70± 1.25^{bd}	0.119± 0.012^{bd}
A1Q1	246.5± 14.6	410.6± 29.0^{b}	2.46± 0.43^{bd}	20.22± 1.69^{bd}	0.122± 0.023^{b}
A1Q2	243.3± 16.2	406.8± 34.1^{b}	2.60± 0.28^{b}	21.34± 1.45^{ab}	0.122± 0.011^{b}
A2Q0	246.5± 16.7	384.6± 39.1^{b}	2.09± 0.45^{c}	18.96± 1.30^{cc}	0.110± 0.017^{bc}
A2Q1	246.4± 15.8	374.7± 32.1^{b}	1.93± 0.37^{c}	18.98± 2.12^{c}	0.103± 0.021^{c}
A2Q2	246.4± 19.2	385.9± 24.0^{b}	2.15± 0.26^{cd}	19.96± 1.52^{dc}	0.108± 0.011^{cd}

1) Values shown are the mean± S.D.(n=10)

2) Values with a common superscript letter within the same column are not significantly different($p<0.05$).

2. Myocardial ultrastructural changes

The results from the ultrastructural studies using transmission electron microscopy are presented in Fig. 1. The control rats fed the basal diet showed the well-preserved subcellular structure(Fig. 2A). In rats receiving lower dose of ADR, the fine structural detail revealed mild seperation of intercalated discs and vacuolization in relation to mitochondria(Fig. 2Ba). In another lesion, although there was focal myofiber degeneration, intercalated discs appeared intact(Fig. 2Bb). Myocardical lesion observed in rats receiving higher dose of coenzyme Q_{10} prior to ADR treatment showed preservation of the subcellular organelles compared to that in A1Q0 group. In addition, there were the relaxed myofibrils and a swollen endothelium of capillary, but this change was in a normal range(Fig. 2C). Mitochondria observed in the group treated with ADR alone were swollen and their inner cristae network was partially disrupted. Sarcolemma was blistered like protrusion as well as occasional loss of myofibrils(Fig. 2D). The myocyte was well preserved in A2Q2 group, but partially relaxed myofibrils were apparent. Swollen endothelium of capillary was observed(Fig. 2 E).

Although the prevention of ADR-induced heart damage was remarkable in coenzyme Q_{10}-fed groups, some alterations such as swollen endothelium of capillary were still present. Supplementation with coenzyme Q_{10} prior to ADR administration prevented to some extent the occurrence of morphologic changes observed in ADR-treated animals. Similarly, in order to prevent the occurrence of cardiomyopathy during the therapy with ADR, several methods using antioxidants have been investigated [1)5)]. Dimitrove et al[1)] indicated that ADR-induced cardiotoxicity could be prevented by selenium. They expected a possible interaction between these two agents. And they reported that no information is available regarding formation of ADR-selenium conjugates, but such a possibility should be considered. Myers et al[5)] also reported that prior treatment with tocopherol significantly decreased the ADR-induced cardiomyopathy, and confirmed it by means of electron microscopy. On the other hand, failure to alter the incidence of severity of cardiac damage in rabbits and dogs was reported[19)].

It is considered that ultrastructural alterations of myocardial cell nuclei may represent an important aspect of ADR toxicity. Merski et al[20)] reported that ultrastructural studies of the effect of ADR on liver and cardiac cell nucleoli of the rat showed the nucleolar segregation. And they suggested that the effects of ADR on the ultrastructure of cardiac cell nucleoli of the rat correlate well with its known inhibition of RNA synthesis. But nuclear alterations were not observed in this study. Therefore these nuclear changes appear to be related mainly to severe ADR toxicity. These findings support the theory that the histologic changes observed in the present study are a result of free radicals released after administration of ADR.

The volume density as well as the surface and numerical density of mitochondria was assessed by electron microscope morphometrics as shown in Table 3. Myocardial mitochondria were not generally affected by lower dosage of ADR administration. However, rats treated with higher dose of ADR exhibited distinct quantitative decrease in mitochondria. An extensive destruction of mitochondrial membranes appeared to lead to the disappearance of mitochondria. Volume density of mitochondria was also significantly decreased, while surface density of mitochondrial innermembrane was not greatly decreased under this condition. But surface density of mitochondrial outermembrane was significantly lowered in higher dose groups of ADR. This decrease was modified by higher level of coenzyme Q_{10} supplementation. In A2Q2 group, volume and

Table 3. Characteristic morphometric indices of rat heart mitochondria

Group	Vv (μm^3)	ISv (μm^2)	OSv (μm^2)	Nv
C	0.444 ± 0.066^{a}	11.32 ± 1.55^{a}	1.94 ± 0.27^{a}	0.457 ± 0.085^{a}
A1Q0	0.415 ± 0.048^{ac}	11.46 ± 1.08^{a}	1.82 ± 0.35^{ac}	0.425 ± 0.082^{ac}
A1Q1	0.415 ± 0.046^{ac}	11.65 ± 2.05^{a}	1.99 ± 0.23^{a}	0.439 ± 0.076^{ac}
A1Q2	0.446 ± 0.045^{a}	11.38 ± 1.38^{a}	1.93 ± 0.32^{a}	0.443 ± 0.045^{ac}
A2Q0	0.353 ± 0.090^{b}	10.62 ± 2.92^{a}	1.52 ± 0.45^{b}	0.328 ± 0.091^{b}
A2Q1	0.391 ± 0.067^{bc}	10.62 ± 1.76^{a}	1.65 ± 0.56^{bc}	0.339 ± 0.076^{bd}
A2Q2	0.450 ± 0.094^{a}	11.90 ± 2.79^{a}	1.82 ± 0.35^{ac}	0.400 ± 0.138^{cd}

1) Vv ; Volume density of mitochodria(mitochondrial volume per $1\mu m^3$ tissue)
ISv ; Surface density of mitochondrial innermembrane(mitochondrial innermembrane surface per $1\mu m^3$ tissue)
OSv; Surface density of mitochondrial outermembrane(mitochondrial outermembrane surface per $1\mu m^3$ tissue)
Nv ; Numerical density of mitochondria(mitochondrial number per $1\mu m^3$ tissue)
2) Values shown are the mean± S.D.(n=30)
3) Values with a common superscript letter within the same column are not significantly different($p<0.05$).

surface density of mitochondria were largely restored to control range.

In conclusion, ADR-treated rats revealed myocardial ultrastructural alterations such as mitochondrial swelling and dilation of the sarcoplasmic reticulum. But these structural changes were somewhat reduced by dietary supplementation of coenzyme Q_{10}.

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=국문초록=

식이 중의 Coenzyme Q_{10} 첨가가 Adriamycin을 투여한 흰쥐의 체내 지질과산화에 미치는 영향
- III. 심근 미세구조 변화에 미치는 영향 -

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식이 중에 첨가된 coenzyme Q_{10}이 ADR을 투여한 흰쥐의 심근 미세구조 변화에 미치는 영향을 검토하기 위하여 ADR 2수준(1.0 및 2.0mg/kg B.W/week)과 coenzyme Q_{10} 3수준(0, 0.1 및 0.5g/kg diet)에 의한 6개의 실험군과 basal diet만을 공급하는 대조군을 설정하여 8주간 실험동물을 사육하였다. 체중증가량은 ADR의 투여수준이 높을수록 유의적으로 감소되었으나 coenzyme Q_{10}급여에 의한 회복효과는 나타나지 않았다. 전자현미경을 통한 관찰결과, ADR 투여는 심근세포내 미세구조의 변성을 유도함이 확인되었고 특히 mitochondria를 비롯한 세포소기관의 소실 및 파괴를 관찰할 수 있었으나 coenzyme Q_{10}을 미리 급여한 군에서는 그 정도가 완화되었다.

LEGENDS

Fig. 1. Transmission electron micrographs of myocardium from coenzyme Q_{10} administration prior to ADR treatment(×12,000)

A. Control group ; Myofibers contain straplike myofibrils with prominent Z bands at regular intervals. Mitochondria (M) are interspersed in regular rows between the fibrils. ID ; Intercalated disc, Sar ; Sarcolemma, N ; Nucleus.

B. A1Q0 group ; (a) Mild separation of the intercalated disc is seen. There is separation of the fasciated adherences of the intercalated discs, but gap junction appears intact. Occasional vacuoles are prominent in relation to the mitochondria(arrow).

(b) The degenerative myocyte contains disrupted cellular remnants(arrow) and vacuoles in relation to the mitochondria. Parallel arrays of Z bands are lost, but intercalated disc appears intact.

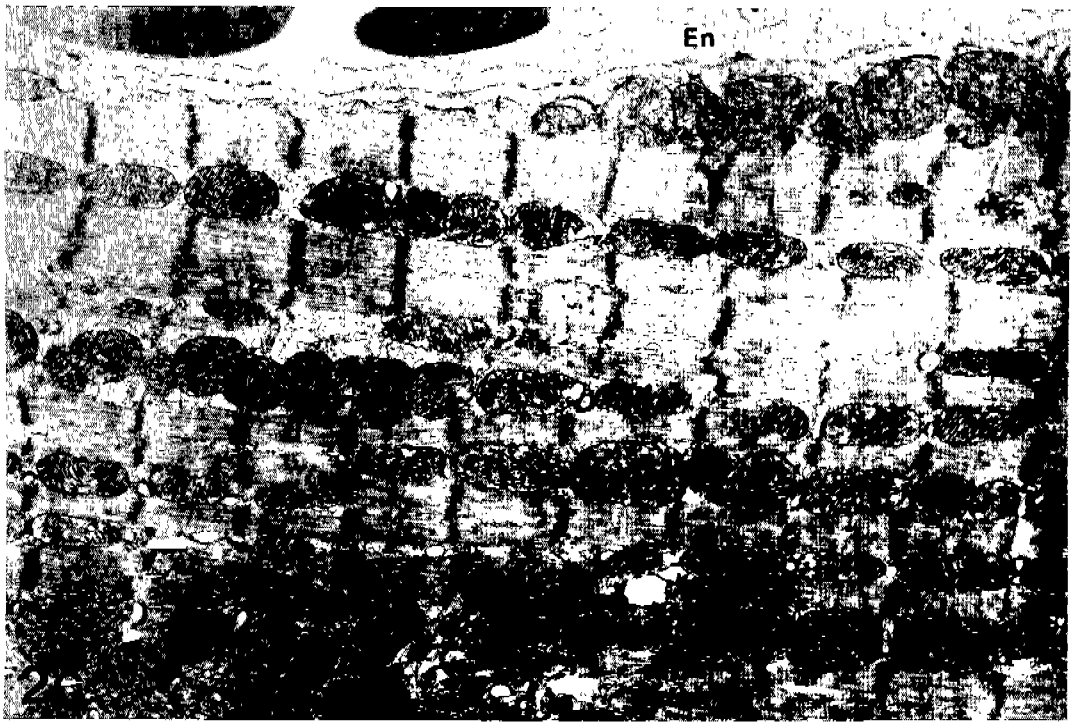
C. A1Q2 group ; The myocytes are within the limits of normal. But capillary is still lined by swollen endothelium.

D. A2Q0 group ; Mitochondria are swollen and their inner cristae are partially disrupted. Occasional loss of myofibrils is seen. Sarcolemma is blistered like protrusion(arrow).

E. A2Q2 group ; The myocyte is well prescrved, but partially relaxed myofibrils (arrow) and swollen endothelium of capillary are seen.

□ 서정숙 · 한인규 논문 사진 부도 ①□

□ 서정숙 · 한인규 논문 사진 부도 ②□



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