Anti-Oxidative Effects of Vitamin B$_2$-Butyrate on the Cardiac Mitochondrial Disorders Induced by Adriamycin

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Summary The combined use of CoQ$_{10}$ with adriamycin has been recommended for reduction of the cardiotoxicity that occurs during cancer chemotherapy. Vitamin B$_2$-butyrate was also investigated in order to determine anti-oxidative effects on adriamycin cardiotoxicity. This vitamin analysis prevented enhanced lipid peroxidation and rectified the respiratory disorders of heart mitochondria induced by adriamycin, however, the deficiency of the CoQ$_{10}$-pool was not rectified. The combined approach of using CoQ$_{10}$ for rectifying the deficiency of this component and of using B$_2$-butyrate for reducing lipid peroxidiation was indicated for adriamycin cancer chemotherapy.

Key Words vitamin B$_2$, adriamycin, mitochondria, lipid peroxide, CoQ$_{10}$

Adriamycin (ADM) is presently one of the most widely used agents for the treatment of solid tumors. Unfortunately, its clinical use has been compromised by an usual and potentially lethal cardiac toxicity. The cardiotoxicity of ADM is known, at least in part, to be mediated through adverse effects on mitochondrial function (3). In our previous paper (6), administration of ADM induced the uncoupling of oxidative phosphorylation and lipid peroxide formation in cardiac mitochondria. High-pressure liquid chromatographic analysis revealed that coenzyme Q$_{10}$ (CoQ$_{10}$) content decreased markedly in cardiac mitochondria of rats treated with ADM. The administration of exogenous CoQ$_{10}$ was able to restore CoQ$_{10}$-levels to normal, relieving respiratory disorders and preventing lipid peroxide formation in cardiac mitochondria. On the contrary, an excess of a single administration of CoQ$_{10}$ tends to enhance lipid peroxidation, owing to the quinone structure within the CoQ$_{10}$ molecule (2). In order to reduce the lipid peroxide formed...
by ADM-treatment, it appeared advantageous to add a structurally different antioxidant with CoQ<sub>10</sub>. Riboflavin-2',3',4',5'-tetrabutyrate (B<sub>2</sub>-butyrate) was selected for this study.

MATERIALS AND METHODS

Adriamycin was purchased from Kyowa Hakko Kogyo Company, Ltd. (Tokyo) and was dissolved in distilled water before use. Riboflavin-2',3',4',5'-tetrabutyrate (B<sub>2</sub>-butyrate) was supplied by Tokyo Tanabe Company Ltd. (Tokyo). B<sub>2</sub>-Butyrate was dissolved in 10% ethanol, and diluted with olive oil until a final concentration of 0.8% B<sub>2</sub>-butyrate was achieved. 2,3,6-Trimethyl-5-nonaprenyl-1,4-benzoquinone (TQ-9) was used for the purpose of quantitative determination of coenzyme Q (CoQ) homologues as an internal standard in high-pressure liquid chromatographic analysis (10).

Four groups of ten male rats were used, the first group daily receiving intraperitoneally ADM (4 mg/kg body weight) alone for one week. The second group received an intraperitoneal injection of B<sub>2</sub>-butyrate (20 mg/kg body weight) daily. The third group received both ADM and B<sub>2</sub>-butyrate. The last batch of animals served as controls.

The animals were killed by decapitation on the 7th day following administration of ADM and/or B<sub>2</sub>-butyrate. Mitochondria were isolated from heart tissue by differential centrifugation after Nagarse digestion (6, 7). A Clark oxygen electrode was used to measure oxygen utilization polarographically using succinate as substrate (8). Respiratory responses are represented as State 3 and State 4 respiration, respiratory control ratio (RCR) and ADP/O ratio. Lipid peroxide content was determined using the thiobarbituric acid-acetic acid reaction, and expressed as nanomoles malondialdehyde per mg protein (5). CoQ homologues were extracted from cardiac mitochondria using n-hexane after saponification, and analyzed by a method of high-pressure liquid chromatography on a LiChrosorb RP-18 column as described in our previous paper (12). The protein content of the mitochondrial suspension was determined using the biuret reaction (1).

RESULTS

The lipid peroxide content of mitochondria from non-treated control animals and from ADM and/or B<sub>2</sub>-butyrate treated animals is shown in Fig. 1. The administration of ADM resulted in the increase of lipid peroxide content in cardiac mitochondria (p < 0.01). The lipid peroxide content in mitochondria from rats treated with ADM and B<sub>2</sub>-butyrate was not as high as that in mitochondria from animals treated with ADM alone. The combined use of B<sub>2</sub>-butyrate with ADM significantly reduced the lipid peroxide formation induced by ADM (p < 0.01).

The respiratory responses of cardiac mitochondria influenced by administration of ADM and/or B<sub>2</sub>-butyrate are shown in Table 1. The administration of
Fig. 1. Lipid peroxide content of cardiac mitochondria from rats treated with adriamycin (4 mg/kg) and/or B₂-butyrate (20 mg/kg) intraperitoneally daily for one week. Lipid peroxide contents are expressed as nanomoles of malondialdehyde per mg of mitochondrial protein.

Table 1. Influence of vitamin B₂-butyrate on respiratory response of cardiac mitochondria of rats treated with adriamycin.

| Animals                      | Oxygen consumption b | RCR c | ADP/O d |
|------------------------------|----------------------|-------|---------|
|                              | State 3 | State 4 |         |         |
| Non-treated control          | 210.6 ± 12.7 | 46.9 ± 2.2 | 4.50 ± 0.45 | 1.51 ± 0.09 |
| Adriamycin                   | 164.5 ± 8.4 | 48.9 ± 7.4 | 3.43 ± 0.57 | 1.42 ± 0.12 |
| Adriamycin and B₂-butyrate   | 189.2 ± 12.4 | 44.5 ± 4.1 | 4.26 ± 0.14 | 1.49 ± 0.11 |
| B₂-butyrate                  | 228.9 ± 2.6 | 53.0 ± 2.0 | 4.31 ± 0.13 | 1.39 ± 0.09 |

a Respiratory responses of cardiac mitochondria from non-treated control and from rats treated intraperitoneally with adriamycin (4 mg/kg body wt.) and/or B₂-butyrate (20 mg/kg body wt.) daily for one week. Ten male rats were used in each group. b The values given for oxygen consumption are expressed as nanoatoms of oxygen utilized per min per mg mitochondrial protein, using succinate as substrate. c RCR (respiratory control ratio) is the quotient of the State 3 respiration value and the succeeding State 4 respiration value. d ADP/O ratio is the molar ratio of the ADP added to the consumed oxygen in State 3.

ADM resulted in a significant decrease in State 3 respiration ($p<0.01$). It is clear from statistical calculation that the combined use of B₂-butyrate with ADM elevated State 3 oxygen consumption more than that with ADM administration alone ($p<0.01$). ADM administration gave a lower value in RCR than with non-treatment ($p<0.05$). Using B₂-butyrate, the RCR value increased significantly ($p<0.05$) toward a normal level. In the animals co-treated with ADM and B₂-butyrate, the respiratory responses were close to normal. The combined use of B₂-butyrate with ADM appears to reduce the uncoupling of oxidative phosphorylation.
Table 2. Content of CoQ homologues in cardiac mitochondria of rats treated with adriamycin and vitamin B$_2$-butyrate.$^a$

| Animals                     | CoQ$_8$     | CoQ$_9$     | CoQ$_{10}$  |
|-----------------------------|-------------|-------------|-------------|
| Non-treated control         | 0.40 ± 0.05 | 9.56 ± 0.45 | 0.54 ± 0.07 |
| Adriamycin                  | 0.43 ± 0.12 | 8.56 ± 1.32 | 0.41 ± 0.06 |
| Adriamycin and vitamin B$_2$-butyrate | 0.45 ± 0.03 | 9.31 ± 0.75 | 0.47 ± 0.04 |
| Vitamin B$_2$-butyrate       | 0.41 ± 0.08 | 10.10 ± 0.95| 0.59 ± 0.05 |

$^a$CoQ$_8$, CoQ$_9$, and CoQ$_{10}$ content in cardiac mitochondria of rats treated with adriamycin (4 mg/kg) and/or B$_2$-butyrate (20 mg/kg) intraperitoneally daily for one week. $^b$CoQ homologues from cardiac mitochondria were analyzed using a method of high-pressure liquid chromatography on a column of LiChrosorb RP-18 (4.5 × 150 mm) using ethanol : water (97:3 v/v) as mobile phase at a flow rate of 0.5 ml/min. The effluent was monitored at 257 nm. 2,3,6-Trimethyl-5-nonaprenyl-1,4-benzoquinone was used as internal standard. Values for CoQ homologues are expressed as $\mu$g per mg protein and represent the average ± SD of 5 determinations.

The CoQ homologue content of cardiac mitochondria influenced by administration of ADM and/or B$_2$-butyrate is shown in Table 2. It is apparent that administration of ADM resulted in a significant decrease in CoQ$_{10}$ content ($p<0.01$). No significant difference was observed in CoQ$_{10}$ content between ADM-treated animals and B$_2$-butyrate co-treated animals ($p>0.05$). Administration of B$_2$-butyrate was not effective in restoring the deficiency of CoQ$_{10}$ content in the cardiac mitochondria of rats treated with ADM.

**DISCUSSION**

We have shown that administration of ADM causes a significant deficiency of the CoQ$_{10}$-pool in rat cardiac mitochondria and involves a mitochondrial dysfunction (6). The therapeutic usefulness of CoQ$_{10}$ for preventing or minimizing the side-effect of cardiotoxicity induced by ADM was confirmed (6). In the recent finding of Myers et al. (4), the deleterious actions of ADM raised the possibility of at least two mechanisms of tissue damage: one which involves lipid peroxidation resulting in cardiac toxicity; another which involves the binding of ADM to the DNA molecule.

In our previous paper (6), the co-administration of exogenous CoQ$_{10}$ with ADM was confirmed to minimize lipid peroxidation in mitochondria and to relieve the respiratory disorders induced by ADM. Takeshige et al. (13) also demonstrated that reduced CoQ$_{10}$ functions as a potent antioxidant against membrane lipid peroxidation in submitochondrial particles. On the contrary, Boveris et al. (14)
documented that a reduced form of CoQ\textsubscript{10} produces small amount of superoxide anione which serves as an initiating factor of lipid peroxidation. Although Takeshige et al. (13) ruled out the direct possibility of generating superoxide anion by antioxidation of CoQ\textsubscript{10}, the site of production is considered as being a region between mercury- and rotenone-sensitive sites in the respiratory chain. In our previous paper (2), it was demonstrated that administration of large amounts of CoQ\textsubscript{10} alone, more than 10 mg per kg body weight (not available for clinical use) for one week, involved enhanced lipid peroxidation in mitochondria. This discrepancy has not yet been resolved, but may be dependent on a quinone functional group of the CoQ\textsubscript{10} molecule. Therefore, the anti-oxidant compound not having quinone structure was studied in the present paper, to determine the anti-oxidative effect under ADM administration. The results reported in this paper indicate that the dose of CoQ\textsubscript{10} administration is only sufficient for reversing the deficiency of CoQ\textsubscript{10} induced by ADM.

Adriamycin contains both quinone and hydroquinone functional groups, suggesting possible activity in oxidation-reduction reactions. It is thought that such a process might be involved in the interaction of ADM with CoQ\textsubscript{10}. This interaction would make complicated the understanding of the mechanism of the anti-oxidative effect of CoQ\textsubscript{10} against ADM.

It is well known that riboflavin (vitamin B\textsubscript{2}) has an anti-oxidant role (15). B\textsubscript{2}-Butyrate is a long-acting vitamin because of its hydrophobic structure (11). The anti-oxidative effect of B\textsubscript{2}-butyrate for lipid peroxidation in ADM administration was found to be more effective than that of the B\textsubscript{2}-free form or CoQ\textsubscript{10} (9). In our present paper, B\textsubscript{2}-butyrate was able to restore lipid peroxide formation induced by ADM administration, and consequently the respiratory dysfunction was improved. However, the CoQ\textsubscript{10}-pool in cardiac mitochondria was deficient, unlike the case of CoQ\textsubscript{10} administration. CoQ is one of the biologically active anti-oxidative compounds, and the anti-oxidative effect of CoQ\textsubscript{10} has been confirmed in our ADM experiment (6). However, for preventing lipid peroxidation induced by excess of CoQ itself, the combined use of B\textsubscript{2}-butyrate with CoQ\textsubscript{10} has been recommended for minimizing the enhanced lipid peroxidation induced by ADM. CoQ is essential for reversing the deficiency of the CoQ-pool, and B\textsubscript{2}-butyrate is effective for reducing lipid peroxide formation. Additional work is in progress to determine the dose rate for CoQ\textsubscript{10} and B\textsubscript{2}-butyrate in administration (2). The combined use of anti-oxidant with CoQ\textsubscript{10} might be of therapeutic benefit for preventing side-effects in ADM cancer chemotherapy.

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