Effect of Riboflavin-Butyrate on Cardiac Glutathione Reductase Affected by Adriamycin

Yuji HINO, Seung Boo YOO, Kiminori KAJIYAMA, Akihiro KAGIYAMA, and Ryohei OGURA
Department of Medical Biochemistry, Kurume University School of Medicine, Asahi-machi, Kurume 830, Japan
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Summary Male Wistar rats received intraperitoneal injections of adriamycin (4 mg/kg body weight/day) and/or riboflavin-butyrate (20 mg/kg body weight/day) for 6 consecutive days. Cardiac mitochondria were then prepared for our present experiment. The combined use of riboflavin-butyrate with adriamycin was evaluated for reduction of lipid peroxide formation in rat cardiac mitochondria. In order to find the mechanism of the effect of riboflavin-butyrate, the glutathione peroxidase-glutathione reductase system was examined. Adriamycin reduced the glutathione reductase activity in rat cardiac mitochondria, but did not affect the glutathione peroxidase activity. The mitochondrial content of flavin adenine dinucleotide, a prosthetic group of glutathione reductase, was greatly reduced and apoprotein of glutathione reductase also decreased. The administration of riboflavin-butyrate with adriamycin increased flavin adenine dinucleotide and glutathione reductase activity. These results suggest that exogenous administration of riboflavin-butyrate is capable of reducing lipid peroxide by both enzymatic detoxification through glutathione reductase and non-enzymatic detoxification due to direct reaction with lipid peroxide.

Key Words riboflavin, adriamycin, mitochondria, lipid peroxide, glutathione reductase, flavin adenine dinucleotide

Adriamycin (ADR) is one of the most widely used agents for the treatment of solid tumors. Its clinical usefulness has been greatly limited by typical and potentially lethal cardiac toxicity. The cardiotoxicity of ADR is known, at least in part, to be mediated through the disordering of mitochondrial function (\textsuperscript{(1)}) In a previous paper (\textsuperscript{(2)}) we reported that administration of ADR to rats induced the uncoupling of oxidative phosphorylation, and an increase of radical formation and
lipid peroxide formation in cardiac mitochondria. The administration of antioxidants such as coenzyme Q_{10} (CoQ_{10}) or riboflavin-butyrate with ADR was able to restore the respiratory function and reduced the lipid peroxide content of cardiac mitochondria to normal levels. All these findings support the working hypothesis that the main side-effect of ADR may derive from a lipid peroxidation process. Detoxification of lipid peroxide in mitochondria is thus a subject of considerable interest. The peroxide detoxification mechanisms of cardiac mitochondria have not been examined, especially with regard to the glutathione peroxidase-glutathione reductase system. In the present study, a beneficial effect of riboflavin-butyrate against adriamycin-induced lipid peroxidation in cardiac mitochondria was observed.

MATERIALS AND METHODS

Adriamycin was supplied by Kyowa Hakko Kogyo Company, Ltd. (Tokyo) and dissolved in distilled water before use. Butyated riboflavin (riboflavin-2',3',4',5'-tetrabutyrate) (B_{2}-butyrate) was supplied by Tokyo Tanabe Company Ltd. (Tokyo). Riboflavin-butyrate was dissolved in 10% ethanol and diluted with olive oil to a final concentration of 0.8% B_{2}-butyrate.

Male Wister rats weighing about 200 g received intraperitoneal injections of ADR (4 mg/kg body weight/day) and/or B_{2}-butyrate (20 mg/kg body weight/day) for 6 consecutive days. On the 7th day, the animals were killed by decapitation. Mitochondria were isolated from cardiac tissue by differential centrifugation after Nagarse digestion (2, 3).

Lipid peroxide content was determined using the thiobarbituric acid-acetic acid reaction, and expressed as nanomoles malondialdehyde per mg protein (4). The activity of glutathione reductase was determined according to the method of Staal et al. (5). Activity coefficient of glutathione reductase by FAD was measured by applying the method of Ono and Hirano (6). The activity of glutathione peroxidase was determined by using the method of Paglia and Valentine (7). Flavin derivatives were further analyzed by high-pressure liquid chromatography (HPLC) on a Lichrosorb RP-2 column using 10% methanol and 10 mM NaH_{2}PO_{4} as the mobile phase, by applying the method of Ohkawa and Ohishi (8). Protein content of the mitochondrial suspension was determined using the biuret reaction (9).

RESULTS

The lipid peroxide content of cardiac mitochondria from control and from ADR and/or B_{2}-butyrate treated animals is shown in Fig. 1. The lipid peroxide content of mitochondria from rats treated with ADR and B_{2}-butyrate is not as high as that of mitochondria from animals treated with ADR alone, and is close to the normal range.

Glutathione peroxidase activity in mitochondria from control animals and...
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 6 days. Lipid peroxide contents are expressed as nmol of malondialdehyde per mg of mitochondrial protein (n=8). *p<0.01 (from control).

Table 1. Effect of adriamycin on glutathione peroxidase activity in rat cardiac mitochondria.
Glutathione peroxidase activity was assayed by using tert-butyl hydroperoxide as a substrate and expressed as nmol NADPH oxidized per min per mg protein. (n=6)

<table>
<thead>
<tr>
<th>Glutathione peroxidase activity</th>
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<tbody>
<tr>
<td>Control</td>
<td>61.5±20.9</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>52.5±12.7</td>
</tr>
</tbody>
</table>

* p<0.01 (from control).

Table 2. Influence of riboflavin-butyrate on glutathione reductase activity of cardiac mitochondria of rats treated with adriamycin.
Glutathione reductase activity was expressed as nmol NADPH oxidized per min per mg protein of mitochondria. (n=8)

<table>
<thead>
<tr>
<th>Glutathione reductase activity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.93±0.38</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>3.68±0.69*</td>
</tr>
<tr>
<td>Adriamycin and riboflavin-butyrate</td>
<td>8.86±2.66</td>
</tr>
<tr>
<td>Riboflavin-butyrate</td>
<td>10.60±1.03</td>
</tr>
</tbody>
</table>

* p<0.01 (from control).

from ADR-treated animals is shown in Table 1. The glutathione peroxidase activity in cardiac mitochondria was not affected by ADR administration.

Glutathione reductase activity in mitochondria from control animals and from ADR and/or B2-butyrate treated animals is shown in Table 2. The administration of ADR resulted in the decrease of glutathione reductase activity in cardiac mitochon-
Table 3. FAD-induced stimulation of glutathione reductase in vitro in rat cardiac mitochondria treated with ADR. (n=5)

<table>
<thead>
<tr>
<th>Activity coefficient (AC)(^a)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Adriamycin</td>
</tr>
</tbody>
</table>

\(^a\) The activity coefficient (AC) of glutathione reductase by FAD is shown in the following equation.

\[
\text{Reduction of absorbance in the presence of FAD (10 min)} \div \text{Reduction of absorbance in the absence of FAD (10 min)} = AC
\]

Table 4. Content of flavin derivatives in cardiac mitochondria of rats treated with adriamycin and/or riboflavin-butyrate.

FAD, FMN, and FR content in cardiac mitochondria of rats treated with adriamycin (4 mg/kg) and/or riboflavin-butyrate (20 mg/kg) intraperitoneally daily for 6 days. Flavin derivatives from cardiac mitochondria were analyzed using a method of high-pressure liquid chromatography on a column of Lichrosorb RP-2 using 10% methanol and 10 mM NaH\(_2\)PO\(_4\) as mobile phase. (n=5)

<table>
<thead>
<tr>
<th>Animals</th>
<th>Flavin derivatives (nmol/mg protein)</th>
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<tbody>
<tr>
<td></td>
<td>FAD</td>
</tr>
<tr>
<td>Control</td>
<td>0.665 ± 0.148</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>0.421 ± 0.106*</td>
</tr>
<tr>
<td>Adriamycin and riboflavin-butyrate</td>
<td>0.715 ± 0.130</td>
</tr>
<tr>
<td>Riboflavin-butyrate</td>
<td>0.896 ± 0.084</td>
</tr>
</tbody>
</table>

\(* p < 0.05\) (from control).

dria (p < 0.01). The glutathione reductase activity in cardiac mitochondria from rats treated with ADR and B\(_2\)-butyrate was compared with that from rats treated with ADR alone. The combined use of B\(_2\)-butyrate with ADR significantly raised the glutathione reductase activity affected by ADR. The activity coefficient of glutathione reductase of cardiac mitochondria by FAD is shown in Table 3. There was no significant effect of FAD on glutathione reductase activity by addition in vitro in cardiac mitochondria treated with ADR.

The flavin derivatives in cardiac mitochondria of rats treated with ADR and/or B\(_2\)-butyrate were analyzed by HPLC and are shown in Table 4. It is apparent that administration of ADR induced a significant decrease in FAD content. A significant difference was observed between ADR-treated animals and B\(_2\)-butyrate co-treated animals in the FAD content of cardiac mitochondria. Administration of B\(_2\)-butyrate was effective in maintaining FAD content in cardiac mitochondria in rats treated with ADR.
DISCUSSION

There are many reports which cover the subject of cardio-selective toxicity (10), however, the exact mechanism of the cardiotoxicity induced by ADR has not been clarified. In the recent findings of Myers et al. (11), the deleterious actions of ADR raise 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 ADR to the DNA.

In our previous study, Ogura et al. (12) demonstrated the marked increase of lipid peroxide, respiratory disorders and decrease of CoQ10 content in mitochondria treated with ADR, accompanied by morphological changes in mitochondria. Combined use of riboflavin-butyrate with ADR was found to prevent enhanced lipid peroxidation and to rectify the respiratory disorders of cardiac mitochondria. Flavin adenine dinucleotide (FAD), is known as a prosthetic group of glutathione reductase. Therefore, the peroxide detoxification mechanism of cardiac mitochondria was examined with regard to the glutathione peroxidase-reductase system. As Doroshow et al. (13) suggested, the glutathione peroxidase-reductase system is the main endogenous defense mechanism against lipid peroxide in the heart. It is important to investigate how these enzymes are affected by ADR. In our present experiment, cardiac mitochondrial glutathione peroxidase was not affected by ADR. However, Doroshow et al. (13) reported that the decrease of cytosolic glutathione peroxidase activity by adriamycin administration or selenium-deficiency, significantly enhanced adriamycin toxicity in the heart. Furthermore Katki and Myers (14) described that the cytosolic glutathione peroxidase activity decreased but that the mitochondrial glutathione peroxidase activity slightly increased in heart muscle of mice kept on a selenium-free diet. These glutathione peroxidases may have different sensitivities to adriamycin depending on their localization in the cytosol and mitochondria. On the other hand, the activity of mitochondrial glutathione reductase decreased remarkably after exposure to ADR. From these results, it is suggested that glutathione reductase is more sensitive to the ADR-induced disorders than is glutathione peroxidase. Glutathione reductase requires FAD as its prosthetic group, so we determined whether or not ADR had an effect on FAD content in rat cardiac mitochondria. Our results confirmed that the decrease of glutathione reductase activity following administration of ADR could be prevented by co-administration of riboflavin-butyrate with ADR, as reflected by the increase of FAD in cardiac mitochondria. Concerning the approximately 2-fold increase in glutathione reductase activity in the B2-butyrate treated rats, similar profiles have been observed by several investigators.

Ono and Hirano (15) postulated the stimulation of protein synthesis following FAD treatment, since the uptake of [3H]uridine into rats lens RNA was increased by FAD addition. Also, Partridge et al. (16) and Cha and Heine (17) demonstrated that the administration of butylated, hydroxytoluene resulted in an increase in glutathione reductase activity of about 2-fold as the result of stimulation of enzyme
protein synthesis in rat liver. We are further attempting to examine the effect of riboflavin-butyrate on the glutathione reductase activity of cardiac mitochondria. We have not yet reached definite conclusions on the mechanism of decrease of FAD content caused by ADR, but it is considered that FAD reacts directly with active oxygen and lipid peroxide as reported by Yagi (18). From our present results, it is suggested that ADR administration causes the structural change of apoprotein of glutathione reductase besides reducing the content of FAD which is a prosthetic group of glutathione reductase. Furthermore, as reported by Burch et al. (19) and Hoppel et al. (20), the disorders of mitochondrial function and morphological changes induced by ADR are similar to those seen in B2-deficiency. The antioxidant role of vitamin B2 has been emphasized. From our present results, riboflavin-butyrate reduces lipid peroxide in cardiac mitochondria affected by ADR. The exogenous riboflavin-butyrate was found to convert to FAD in cardiac mitochondria, the FAD being effective as a prosthetic group for glutathione reductase. In addition to enzymatic detoxification, vitamin B2 has been known to react directly with lipid peroxide non-enzymatically, as mentioned by Ohama and Yagi (21). Additional work is in progress to determine which mechanism of riboflavin-butyrate is essential in reducing lipid peroxide induced by ADR in cardiac mitochondria: the enzymatic process through the detoxification system or a direct antioxidant effect.

REFERENCES

Riboflavin-But. and GR Activity


