Summary The present study was undertaken to determine whether administration of Adriamycin causes the depletion of riboflavin content. Rats received intraperitoneal injections of Adriamycin (4 mg per kg body weight) for 6 consecutive days. Urinary riboflavin excretion began to increase after 2 days of treatment with Adriamycin. Erythrocyte FAD levels decreased gradually and plasma lipid peroxide contents increased markedly at the 6th day. The activity coefficient of erythrocyte glutathione reductase showed a significant increase before the decrease of flavin content and the elevation of lipid peroxide level. Therefore, the value of this coefficient obtained from erythrocyte appears to be a reliable index of riboflavin deficiency, particularly during the early stage.

Key Words riboflavin, Adriamycin, glutathione reductase, lipid peroxide, urinary excretion, heart mitochondria
MATERIALS AND METHODS

Adriamycin was obtained from Kyowa Hakko Kogyo (Tokyo) and dissolved in distilled water before use. Male Wistar rats received intraperitoneal injections of adriamycin (4 mg per kg body weight) daily for 6 consecutive days, to induce an acute cardiotoxicity in a short period of time (6, 7). Blood was obtained from rats at the 3rd, 5th and 7th day, from the cervical vein with a heparin-coated syringe (approximately 1 ml each). Flavin derivatives in the erythrocytes were analyzed by high-pressure liquid chromatography [HPLC] on a Lichrosorb RP-2 column using 10% methanol and 10 mM NaH₂PO₄ as the mobile phase, by the method of Ohkawa and Ohishi (8). Lipid peroxide content was determined by thiobarbituric acid-acetic acid method and expressed as nmol malondialdehyde per 1 ml plasma or mg protein (9). Protein content was determined using biuret reaction (10). The raw urine specimens were collected in a flask assembled with a metabolic cage. Specimens obtained from animals having diarrhea were omitted from the present experiment. The quantitative analysis of flavin in urine was performed by applying the fluorometric method of Yagi (11). The method was based on the fluorescence measurement of lumiflavin which is derived from riboflavin, FMN or FAD by photolysis in alkaline medium and is extracted with chloroform from acidified medium. To remove chloroform-soluble fluorescent substances in urine, urine was pretreated with chloroform until all the chloroform-soluble fluorescent substances were removed. After the preextraction, the photolysis was performed. In addition, chloroform extract from non-irradiated sample solution, was processed in parallel as blank (11). All data were presented as mean value with standard deviation. Statistical significance was tested by Student’s t-test. The criterion of significance was a p value of less than 0.05.

RESULTS AND DISCUSSION

The time course of urinary riboflavin excretion of rats treated with adriamycin is shown in Fig. 1. The urinary excretion began to increase rapidly after 2 days of intraperitoneal injection with adriamycin.

To find the blood level of riboflavin, erythrocyte riboflavin content was determined. As shown in Fig. 1, adriamycin was administered for 6 days, and the total flavin content in the erythrocytes was decreased remarkably at the 6th day, following the increase of urinary excretion. Especially important is the decline of FAD content, as shown in Table 1. However, adriamycin had no significant effect upon FMN and free riboflavin (FR) contents in the erythrocytes. As glutathione reductase requires FAD as its prosthetic group, glutathione reductase activity in erythrocytes was determined. Along with the depletion of total riboflavin in the erythrocytes, glutathione reductase activities fell to half at the 6th day, as shown in Table 1, in parallel with the decrease of FAD content in the erythrocytes.

Fig. 1. Urinary flavin excretion and erythrocyte flavin content of rats treated with adriamycin. Adriamycin was injected intraperitoneally for 6 consecutive days at 4 mg per kg body weight.

Table 1. Erythrocyte flavin derivative content and glutathione reductase activity of rats treated with adriamycin.

<table>
<thead>
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<th>0</th>
<th>2</th>
<th>4</th>
<th>6 days</th>
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<tbody>
<tr>
<td>Erythrocyte flavin¹</td>
<td></td>
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<tr>
<td>FAD</td>
<td>1.30±0.23</td>
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<td>FMN</td>
<td>0.42±0.18</td>
<td>0.45±0.17</td>
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<td>FR</td>
<td>0.14±0.04</td>
<td>0.10±0.07</td>
<td>0.10±0.02</td>
<td>0.13±0.06</td>
</tr>
<tr>
<td>Glutathione reductase²</td>
<td>1.60±0.32</td>
<td>1.38±0.17</td>
<td>1.44±0.33</td>
<td>0.95±0.38*</td>
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<tr>
<td>Activity coefficient</td>
<td>1.26±0.26</td>
<td>1.21±0.24</td>
<td>1.53±0.05*</td>
<td>2.46±0.71*</td>
</tr>
</tbody>
</table>

Adriamycin was injected intraperitoneally for 6 consecutive days at 4 mg per kg body weight. ¹nmol per g Hb (n=5). ²nmol NADPH oxidized per min per mg protein (n=7). *p<0.05 vs. control (0).

Activity coefficients of erythrocyte glutathione reductase were determined by expressing the ratio of the enzyme activity in the presence of exogenous FAD to that in the absence of FAD in vitro. Significant increases in the activity coefficient were found in the erythrocytes obtained on the 4th and 6th day. Thus, the adriamycin administration produces changes in riboflavin homeostasis that are detectable by assays that are utilized to assess riboflavin deficiency.

As it is suggested that the deficiency of riboflavin brings about a decrease of...
Table 2. Glutathione reductase activity and lipid peroxide content in heart mitochondria of rats treated with adriamycin.

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<th>6 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione reductase</td>
<td>7.3±0.5 (8)</td>
<td>6.8±0.4 (5)</td>
<td>7.2±0.7 (5)</td>
<td>5.8±0.8* (8)</td>
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<tr>
<td>Lipid peroxide</td>
<td>0.93±0.21 (5)</td>
<td>0.95±0.008 (4)</td>
<td>1.08±0.07 (6)</td>
<td>1.27±0.13* (6)</td>
</tr>
</tbody>
</table>

Adriamycin was injected intraperitoneally for 6 consecutive days at 4 mg per kg body weight. 1 nmol NADPH oxidized per min per mg protein. 2 nmol malondialdehyde per mg protein. *p < 0.05 vs. control (0).

Glutathione reductase activity and leads to an increase of lipid peroxide (9), serum lipid peroxide content was determined. As shown in Fig. 1, serum lipid peroxide content increased remarkably at the 6th day following administration of adriamycin. It has been known that increased lipid peroxides in the blood exert an influence on the lipid peroxide level in tissues, because the lipid peroxides circulate in the blood stream until they are decomposed by the enzyme system (9). In fact, the TBA reaction positive content of rats' heart increased significantly from 1.13±0.22 to 1.51±0.33 nmol per mg protein at the 6th day. In addition, adriamycin is particularly known to induce mitochondrial damages in the heart (6). Therefore, the effects of adriamycin on heart mitochondria were examined to determine the correlation of flavin depletion in erythrocytes with that in the heart mitochondria. As shown in Table 2, an elevation of lipid peroxide and a decrease of glutathione reductase activity were detected at the 6th day. The FAD content in heart mitochondria at the 6th day was found to have decreased significantly from 0.67±0.15 nmol per mg protein to 0.42±0.11. The changes of FMN and FR content were not significant. In addition, the flavokinase activity in rat heart (2.3 units/mg protein/h) was found to be lower than that in rat liver (29.3 units) by approximately one-tenth. Therefore, the flavin deficiency must have a perceptible influence on FAD content in rat heart.

Pelliccione et al. (3) found that chlorpromazine treatment for 3 weeks to rats accelerated urinary riboflavin loss and tissue depletion of FAD. Among the organs, the heart was particularly sensitive to phenothiazine derivatives. Riboflavin metabolism was inhibited at lower doses in the heart than in other organs (2). A series of phenothiazine derivatives was found by Pinto et al. (2) to inhibit the incorporation of 14C-riboflavin into FAD. A significant increase in the activity coefficient of erythrocyte glutathione reductase was detected in the chlorpromazine-treated animals (2). In addition, Yagi et al. (4, 5) discussed the mechanism of the depletion of riboflavin as a result of the administration of antibiotics. Chloramphenicol was found to inhibit D-amino acid oxidase (flavin enzyme).

It will require a further study to determine what mechanism is involved in the depletion of riboflavin content in erythrocytes and tissues affected by adriamycin.
The following several possibilities may be considered. The adriamycin structure and the isoalloxazine ring of FAD may form an electron donor-acceptor complex, or adriamycin may accelerate the riboflavin metabolism. In addition, adriamycin is known to convert to its semiquinone radical form, which leads to the production of a reactive oxygen species (12). Therefore, riboflavin may be consumed in the scavenging of free radical oxygen species produced by semiquinone-adriamycin.

In conclusion, with respect to heart mitochondrial damages induced by adriamycin, it is worthy of notice that significant increases in the erythrocyte activity coefficient of glutathione reductase are detectable prior to the remarkable increase of serum and tissue lipid peroxide contents. The elevated activity coefficient appears to be a sensitive index of marginal riboflavin deficiency.

REFERENCES


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