Mechanism of the Cardioprotective Effects of Docetaxel Pre-administration Against Adriamycin-Induced Cardiotoxicity

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Abstract. We revealed that pre-treatment with docetaxel (DOC) 12 h before adriamycin (ADR) administration significantly reduced ADR-induced toxic death compared with the simultaneous dosing schedule that was commonly used in previous studies. We considered that pre-treatment with DOC relieves ADR-induced cardiotoxicity. In this study, we investigated the influence of DOC on the pharmacokinetics and pharmacodynamics of ADR in order to clarify the mechanism by which DOC pre-treatment relieves ADR-induced cardiotoxicity. When ADR and/or DOC was intravenously administered, the DOC pre-treatment (DOC-ADR) group showed significantly less toxic death than the ADR-alone group. We examined hepatopathy, nephropathy, leukopenia, and cardiotoxicity, all of which can cause toxic death. Of these toxicities, ADR-induced cardiotoxicity was significantly relieved in the DOC-ADR group. To elucidate the mechanism by which DOC pre-treatment relieved ADR-induced cardiotoxicity, lipid peroxidation as a proxy for the free radical level and the pharmacokinetics of ADR were measured. There was no difference in the pharmacokinetics of ADR between the ADR and DOC-ADR groups. On the other hand, the DOC-ADR group showed significantly inhibited lipid peroxidation in the heart compared with the ADR group. It was considered that DOC pre-administration inhibited ADR-induced free radicals and decreased cardiotoxicity.

Keywords: docetaxel, adriamycin, cardiotoxicity, free radical, pre-administration

Introduction

Metastatic breast cancer (MBC) is almost always incurable (1), and the median survival is on the order of 18 – 24 months (2). Combination therapy with adriamycin (ADR) and docetaxel (DOC) has demonstrated high survival and progression-free survival rates against MBC compared with other therapies (3, 4). However, the combination of ADR and DOC induces severe adverse effects, limiting its clinical use in many patients with MBC (4, 5). Although we previously surveyed the protocols for combination therapy with ADR and DOC, all of the studied protocols were very similar (6 – 8). We thus studied the dosing intervals and dosing sequences of this combination therapy in mice and revealed that the pre-administration of DOC (DOC-ADR), in which DOC was administered 12 h before ADR injection, significantly reduced toxic death compared with the simultaneous dosing (ADR/DOC) group, in which ADR and DOC were administered without any intervals between injections that is commonly used in clinical practice, and the pre-administration of ADR (ADR-DOC) group, in which DOC was administered 12 h after ADR injection. In addition, it was demonstrated that the DOC-ADR group showed an increased survival rate of 95.8% compared with 33.3% in the ADR group, in which ADR alone was administered (9).

The adverse effects of ADR include reversible acute
toxicities such as myelosuppression, nausea, and vomiting, and irreversible chronic toxicities such as the development of cardiotoxicity followed by congestive heart failure (CHF). ADR-induced CHF occurs at a total cumulative ADR dose of 300 mg/m² in humans (10). It is also reported that ADR leads to dose-dependent cardiotoxicity after the administration of a single high dose or the respective administration of low doses in various animals (11–13). In rodents, a cumulative dosage of 15–20 mg/kg depressed cardiac function and produced high mortality (14, 15). When a cumulative ADR dose of 20 mg/kg was administered to mice in our studies, most of the ADR-treated mice died, and the creatine phosphokinase isoenzyme – MB (CPK-MB) level in the ADR group was significantly higher than that in the control group. On the other hand, the DOC-ADR group inhibited the toxic death and the increase in CPK-MB level compared with the ADR group (9). Therefore, we considered that the main cause of toxic death was ADR-induced cardiotoxicity, and the DOC-ADR group relieved the ADR-induced cardiotoxicity.

Although the exact mechanisms underlying ADR-induced cardiac damage are not fully understood, there are two major hypotheses for the mechanism: the iron and free-radical hypothesis and the metabolite hypothesis (16). The iron and free radical hypothesis states that free radicals caused by the iron catalytic redox reaction induce cardiotoxicity. On the other hand, the metabolite hypothesis states that adriamycinol (ADRol), a metabolite of ADR, inhibits the Ca²⁺-Mg²⁺ ATPase of the sarcoplasmic reticulum, the FₐF₁ proton pump of mitochondria, and the Na⁺/K⁺ ATPase and Na⁺/Ca²⁺ exchanger of the sarcolemma, thereby inducing cardiotoxicity (16, 17). The DOC-ADR protocol may inhibit the ADR-induced cardiotoxicity by affecting these mechanisms. In this study, we studied the influence of DOC pre-administration on several toxicities. To elucidate the mechanism by which DOC relieves the ADR-induced cardiotoxicity, we measured the pharmacokinetics of ADR and the free radical level in cardiac tissue.

**Materials and Methods**

**Animals**

Male ICR mice (6 weeks of age) were purchased from Kyudo Co., Ltd. (Saga). The mice were housed at 3 – 4 per cage under standardized light–dark cycle conditions (lights on and off at 7:00 and 19:00, respectively) and allowed free access to food and water. All animal care procedure and experiments were performed in accordance with the Guidelines for Animal Experimentation of Nagasaki University under approval from the Institutional Animal Care and Use Committee.

**Drugs and dosing schedules**

ADR, which was supplied by Kyowa Hakko Kirin Co., Ltd. (Tokyo), was dissolved in saline. DOC (Taxotere) was dissolved in 95% ethanol and diluted with 5% glucose solution. ADR and DOC were intravenously injected. The mice were divided into the DOC pre-administration group (DOC-ADR), in which ADR was administered 12 h after DOC injection; the ADR alone group (ADR), in which ADR was administered alone; and the saline-treated group (control).

**Determination of survival**

To study toxic death, ADR alone or a combination of ADR and DOC (DOC-ADR) was intravenously administered once (20 or 25 mg/kg of ADR with or without 12.5 mg/kg of DOC, n = 8 – 19). Survival time was recorded for 28 or 56 days in each mouse.

**Measurement of leukocyte counts**

ADR alone or a combination of ADR and DOC (DOC-ADR) was intravenously administered once (20 mg/kg of ADR with or without 12.5 mg/kg of DOC, n = 10). Blood samples were withdrawn from the orbital sinus on day 3 after drug administration, and then leukocyte counts were measured.

**Measurement of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and blood urea nitrogen (BUN)**

ADR alone or a combination of ADR and DOC (DOC-ADR) was intravenously administered once (20 mg/kg of ADR with or without 12.5 mg/kg of DOC, n = 7 – 10). Blood samples were withdrawn from the orbital sinus on days 14 and 28 after ADR administration. All blood samples were centrifuged immediately at 3,000 × g for 15 min at 15°C, and then the plasma was frozen at −80°C until the assays were performed. ALT and AST were measured using Transaminase CII-Test Wako (Wako Pure Chemical Industries, Ltd., Osaka). BUN were measured using Urea N B (Wako Pure Chemical Industries, Ltd.).

**Measurement of cardiotoxicity**

To estimate the cardiotoxicity induced by ADR administration, ADR alone or a combination of ADR and DOC (DOC-ADR) was intravenously administered once (20 mg/kg of ADR with or without 12.5 mg/kg of DOC, n = 6 or 7). For electrocardiography (ECG) recordings, conscious mice were restrained by their extremities in tubular plastic holders. On days 0, 7, 14, 21, and 28, each mouse had their QRS, RR, QT, and ST intervals and QTc measured by ECG (Softron Inc., Tokyo).
Histopathology of the heart
To estimate the cardiotoxicity induced by ADR administration, ADR alone or a combination of ADR and DOC (DOC-ADR) was intravenously administered once (20 mg/kg of ADR with or without 12.5 mg/kg of DOC, n = 3). All heart samples were isolated from the mice under ether anesthesia on day 28 after drug administration, and then all heart samples were fixed in 10% neutral buffered formalin for 24 h. The hearts were then trimmed, processed, embedded in paraffin plastic polymer, sectioned at approximately 3 μm, and stained with hematoxylin and eosin (HE) and Masson’s trichrome (MT) for routine histopathology.

Measurement of pharmacokinetics
ADR alone or a combination of ADR and DOC (DOC-ADR) was intravenously administered once (20 mg/kg of ADR with or without 12.5 mg/kg of DOC, n = 3 – 7). Blood samples were drawn from the hearts of the mice under ether anesthesia at 5, 15, 30, 60, 120, 240, 360, 720, and 1,440 min, and heart samples were isolated from the mice under ether anesthesia at 5, 15, 120, and 720 min after ADR had been administered. All blood samples were immediately centrifuged at 880 × g for 15 min, and then the plasma was removed and frozen at −80°C until each assay. All heart samples were immediately rinsed with PBS(−) and frozen at −80°C until each assay. The plasma (300 μL) was mixed with 300 μL of solution buffer [0.01 M phosphate buffer (pH 3.0): methanol (1:1)], 300 μL of internal standard (300 ng/mL epirubicin), 1 mL of Colthoff buffer (pH 8.0), and 8 mL of extraction solvent [ethyl acetate / n-propanol (4:1)]; and the solution was then shaken for 15 min. Then, the hearts of the mice were homogenized with 19 volumes of Colthoff buffer (pH 8.0). The homogenate solutions (2 mL) were mixed with 200 μL of solution buffer, 300 μL of internal standard (300 ng/mL epirubicin), and 8 mL of extraction solvent [ethyl acetate / n-propanol (4:1)]; and the solution was then shaken for 15 min. Samples were centrifuged at 1,190 × g for 5 min, and the organic phase was dried with N2. The resultant residue was resuspended in 300 μL of solution buffer and centrifuged at 15,000 × g for 5 min. The supernatant (200 μL) was injected into a high-performance liquid chromatography system comprising a pump (LC-10AD; Shimadzu, Kyoto), a detector (RF-10A, Shimadzu), and an analytical column (Unison UK-C18, 250 × 4.6 mm; Imtakt, kyoto). The temperature of the column was maintained at 50°C. The mobile phase consisted of 0.01 M phosphoric acid (solvent A) and acetonitrile (solvent B). In the initial conditions, the mobile phase consisted of 75% A and 25% B and was then changed along a linear gradient over 12 min to 66% A and 34% B, before being changed along a second linear gradient over 5 min to 75% A and 25% B. The column effluent was monitored at Ex 470 nm and Em 585 nm.

Measurement of lipid peroxidation
ADR alone or a combination of ADR and DOC (DOC-ADR) was intravenously administered once (20 mg/kg of ADR with or without 12.5 mg/kg of DOC, n = 17). The hearts of the mice were isolated under ether anesthesia 1 h after ADR administration. Lipid peroxidation in myocardial tissue was assayed by measuring the amount of thiobarbituric acid reactive substances (TBARs) using the TBA method (18), and 1,1,3,3-tetraethoxypropane was used as the standard.

Statistics
The survival period was plotted according to the Kaplan-Meier method and compared using the log-rank test. ALT, AST, BUN, leukocyte count, ECG, and drug concentration were recorded as the mean ± standard deviation (S.D.). Groups were compared by one-way analysis of variance (ANOVA), and differences between groups were determined by Scheffe’s test. Differences between two groups were analyzed by the Student’s t-test. Lipid peroxidation was analyzed as non-parametric data. Intragroup post-hoc testing was performed using the Mann-Whitney U-test with Bonferroni’s correction after the Kruskal-Wallis test. A probability level of less than 0.05 was considered to be significant.

Results
Influence of DOC pre-administration on toxic death
The frequency of ADR-induced toxic death was dependent on the dose of ADR administered. Most of the ADR (25 mg/kg)-treated group had died within a month, and the survival rate was 61.1% in the ADR (20 mg/kg)-treated group at two months. The DOC-ADR groups showed significantly higher survival rates than the ADR groups, regardless of the dose of ADR administered (P < 0.05, respectively, Fig. 1).

Influence of DOC pre-administration on hepatopathy, nephropathy, and leukopenia
For the subsequent experiments, we adopted 20 mg/kg as the ADR dose, which showed a higher survival rate at one month, to monitor hepatopathy and nephropathy at regular intervals.

There were no significant differences in ALT or AST levels, high levels of which indicate the presence of hepatopathy, among any of the groups on days 14 and 28 (Fig. 2: A, B, D, and E). On day 14, BUN levels, high levels of which indicate the occurrence of nephropathy, did not differ among any of the groups (Fig. 2C). The
ADR group showed significantly higher BUN levels compared with the control and DOC-ADR groups on day 28 ($P < 0.01$, Fig. 2F). The leukocyte counts were 10,491 ± 2,331 cell/μL in the control group, 2,500 ± 670 cell/μL in the ADR group, and 1,100 ± 313 cell/μL in the DOC-ADR group. The leukocyte counts in the DOC-
ADR and ADR groups were significantly decreased compared with those of the control group (P < 0.01). Also, there was no significant difference of the leukocyte counts between the DOC-ADR and ADR groups. The leukocyte counts of the DOC-ADR group were 44% lower than those of the ADR group.

**Influence of DOC pre-administration on cardiotoxicity**

The QRS, RR, QT, and ST intervals and QTc of the mice were measured on days 0, 7, 14, 21, and 28 after ADR with or without DOC had been administered in the DOC-ADR and ADR groups (Table 1). There was no significant difference in the QRS interval among any of the groups. On days 21 and 28, the RR interval in the ADR group was significantly longer than those in the control and DOC-ADR groups (P < 0.05 and P < 0.01, respectively).

On day 7, the QT and ST intervals and QTc were significantly extended in the ADR group compared with the control group (P < 0.05 and P < 0.01). Moreover, the difference in these parameters increased between the control and ADR groups with the passage of time (P < 0.01, respectively). On the other hand, the extension of the QT and ST intervals and QTc was significantly inhibited in the DOC-ADR group compared with the ADR group on days 21 and 28 (P < 0.05 and P < 0.01, respectively). The DOC-ADR group maintained similar QT and ST intervals and QTc values to those in the control group.

The histopathology of the heart was evaluated on day 28 after ADR with or without DOC had been administered in the DOC-ADR and ADR groups (Fig. 3). In the ADR group, eosinophilic cytoplasm, enrichment of nucleus, and collagen fibers were seen; however, there were no lethal foci. In the DOC-ADR group, mild myocardial necrosis, localized elimination of cardiomyocytes, and hyperplasia of collagen fibers were seen; however, there were no lethal foci (Fig. 3).

**Influence of DOC pre-administration on ADR pharmacokinetics in the plasma and heart**

When the combination of ADR and DOC or ADR alone was administered, the plasma and heart ADR concentrations showed no change between the DOC-ADR and ADR groups. The plasma ADRol concentration in the ADR group was significantly higher than that in Table 1.

### Table 1. ECG value after a single administration of ADR with or without DOC in mice

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
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<tbody>
<tr>
<td><strong>QRS (ms)</strong></td>
<td></td>
<td></td>
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<tr>
<td>control</td>
<td>9.36 ± 1.00</td>
<td>8.67 ± 0.82</td>
<td>9.56 ± 1.19</td>
<td>9.00 ± 0.76</td>
<td>9.44 ± 0.46</td>
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<td>ADR</td>
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<td>8.81 ± 1.09</td>
<td>8.57 ± 1.05</td>
<td>9.10 ± 1.05</td>
<td>8.71 ± 0.59</td>
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<td>DOC-ADR</td>
<td>9.36 ± 1.47</td>
<td>9.11 ± 1.42</td>
<td>8.72 ± 1.14</td>
<td>8.67 ± 1.41</td>
<td>8.50 ± 1.57</td>
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<td><strong>RR interval (ms)</strong></td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>control</td>
<td>96.5 ± 12.2</td>
<td>84.7 ± 10.1</td>
<td>80.2 ± 3.9</td>
<td>78.5 ± 3.5</td>
<td>80.9 ± 4.5</td>
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<tr>
<td>ADR</td>
<td>95.9 ± 17.3</td>
<td>90.4 ± 8.0</td>
<td>83.2 ± 4.7</td>
<td>87.4 ± 3.7**</td>
<td>88.9 ± 4.8*</td>
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<td>DOC-ADR</td>
<td>94.0 ± 12.5</td>
<td>85.3 ± 4.0</td>
<td>81.3 ± 6.6</td>
<td>81.6 ± 1.6</td>
<td>80.9 ± 2.8</td>
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<td><strong>QT interval (ms)</strong></td>
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<tr>
<td>control</td>
<td>23.7 ± 1.2</td>
<td>22.6 ± 3.3</td>
<td>23.4 ± 2.2</td>
<td>22.4 ± 2.5</td>
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<tr>
<td>ADR</td>
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<td>31.3 ± 4.1**</td>
<td>32.5 ± 2.5**</td>
<td>34.4 ± 4.3**</td>
<td>39.9 ± 6.1**</td>
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<tr>
<td>DOC-ADR</td>
<td>23.8 ± 2.4</td>
<td>27.7 ± 4.8</td>
<td>28.1 ± 6.4</td>
<td>26.8 ± 5.0</td>
<td>27.7 ± 5.2</td>
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<td><strong>QTc</strong></td>
<td></td>
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<tr>
<td>control</td>
<td>76.5 ± 5.0</td>
<td>77.7 ± 13.9</td>
<td>82.4 ± 9.3</td>
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<td>ADR</td>
<td>81.5 ± 11.8</td>
<td>103.6 ± 13.7*</td>
<td>112.0 ± 8.1**</td>
<td>115.7 ± 15.1**</td>
<td>133.1 ± 19.6**</td>
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<td>DOC-ADR</td>
<td>77.9 ± 12.4</td>
<td>94.2 ± 16.6</td>
<td>97.7 ± 21.4</td>
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<td>97.0 ± 17.3</td>
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<td></td>
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<tr>
<td>control</td>
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<td>13.9 ± 3.0</td>
<td>13.9 ± 2.0</td>
<td>13.4 ± 2.7</td>
<td>12.2 ± 2.1</td>
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<tr>
<td>ADR</td>
<td>15.7 ± 3.2</td>
<td>22.5 ± 4.2**</td>
<td>23.9 ± 2.5**</td>
<td>25.3 ± 4.5**</td>
<td>31.2 ± 6.4**</td>
</tr>
<tr>
<td>DOC-ADR</td>
<td>14.5 ± 2.0</td>
<td>18.6 ± 4.4</td>
<td>19.3 ± 6.3</td>
<td>18.2 ± 4.9</td>
<td>19.2 ± 5.8</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 6 or 7 mice. *P < 0.05 and **P < 0.01 vs. control. *P < 0.05 and ***P < 0.01 vs. DOC-ADR.
the DOC-ADR group at 60 min after ADR administration ($P < 0.05$, Fig. 4B). The ADR group displayed a significantly lower ADRol concentration in cardiac tissue compared with the DOC-ADR group at 15 min after ADR administration ($P < 0.01$, Fig. 5B). The mean area under the curve (AUC) of plasma ADR was 6,566 ng/mL per hour in the ADR group and 6,374 ng/mL per hour in the DOC-ADR group, and there was no clear difference in AUC between the ADR and DOC-ADR groups. In addition, the mean AUC of plasma ADRol was 1,565 ng/mL per hour in the ADR group and 1,783 ng/mL per hour in the DOC-ADR group, and the AUC of plasma ADRol in the DOC-ADR group was 1.14-fold higher than that in the ADR group.

Influence of DOC pre-administration on lipid peroxidation in myocardial tissue

The TBARs levels in myocardial tissue were significantly higher in the ADR group than those in the control group ($P < 0.05$, Fig. 6). The DOC-ADR group showed a significantly inhibited heart TBARs levels compared with the ADR group ($P < 0.05$), and there was no significant difference between the DOC-ADR and control groups.

Discussion

We demonstrated in our previous study that single and repeated administration of ADR showed similar survival rates, and these in all situations were improved by DOC pre-administration (9). When 20 or 25 mg/kg of ADR and 12.5 mg/kg of DOC were intravenously administered in the present study, the mice in which ADR was administered 12 h after DOC injection showed a higher survival rate than the mice in which ADR was administered alone, as was found in previous studies (9). We consider that the DOC pre-administration improves the ADR-induced toxic death according to the same mechanisms, regardless of single and repeated administration of ADR, and that the model is suitable to clarify the mechanism.

To clarify how DOC pre-treatment reduced the incidence of toxic death, we evaluated the adverse effects of the different regimens such as hepatic and renal dysfunction, leukopenia, and ADR-induced cardiotoxicity. The drug-treated groups showed significantly decreased leukocyte counts compared with the control group, and the DOC-ADR group, which showed a higher survival rate, displayed more severe leukopenia than the ADR group. After monitoring ALT and AST levels for a month, no lethal hepatic dysfunction was observed in any of the
drug-treated groups. Therefore, we considered that neither leukopenia nor hepatic dysfunction had caused the toxic death observed after ADR administration. No nephrotoxicity was observed in the drug-treated groups during the early phase after drug injection. On day 28, renal function aggravation was observed in the ADR group, although the DOC-ADR group displayed normal BUN values. On the other hand, the QT and ST intervals and QTc were significantly extended in the ADR group compared with the control group from day 7, and it was shown that cardiac function worsened in the ADR group. Many reports have shown that ADR administration extends the QT and ST intervals in rodents (12, 19), as did the present study. The QT interval shows the time from the origin of ventricle excitation (depolarization) to the end of ventricle excitation (repolarization). Extension of the QT interval can have many causes such as ischemic heart disease, heart failure, cardiomyopathy, congenital QT extension syndrome, electrolyte disturbance, and drug action. When the QT interval is extended, the risk of torsades de pointes, which causes syncopal attacks and sudden death, is higher (20 – 22). From these results, we thought that the cardiotoxicity was caused by ADR as the first step and renal function was indirectly depressed by decreases in renal blood flow or exacerbation of the rennin-angiotensin system caused by the ADR-induced cardiotoxicity. On the other hand, the DOC-ADR group showed significantly inhibited extension of the RR, QT, and ST intervals and QTc compared with the ADR group; and the control and DOC-ADR groups showed similar values for these parameters. Moreover, we reported that the pre-administration of DOC markedly inhibited the

![Fig. 4](image_url)

**Fig. 4.** Influence of pre-administration of DOC on ADR and ADRol concentrations in plasma at 5, 15, 30, 60, 120, 240, 360, 720, and 1,440 min after a single administration of ADR and/or DOC in mice. ADR (20 mg/kg, i.v.) and/or DOC (12.5 mg/kg, i.v.) were administered in the pre-DOC dosing (triangle, DOC-ADR) and ADR alone (circle, ADR) groups. Each value is the mean ± S.D. The plasma ADR concentrations showed no change between the DOC-ADR and ADR groups (A). The plasma ADRol concentration in the ADR group was significantly higher than that in the DOC-ADR group at 60 min after ADR administration (*P < 0.05) (B).

![Fig. 5](image_url)

**Fig. 5.** Influence of pre-administration of DOC on ADR and ADRol concentrations in cardiac tissue at 5, 15, 120, and 720 min after a single administration of ADR and/or DOC in mice. ADR (20 mg/kg, i.v.) and/or DOC (12.5 mg/kg, i.v.) were administered in the pre-DOC dosing (triangle, DOC-ADR) and ADR alone (circle, ADR) groups. Each value is a mean ± S.D. The cardiac ADR concentrations showed no change between the DOC-ADR and ADR groups (A). The ADR group displayed a significantly low ADRol concentration in cardiac tissue compared with the DOC-ADR group at 15 min after ADR administration (**P < 0.01) (B).
increase in the expression of CPK-MB, a marker of cardiotoxicity, induced by ADR administration (9). Therefore, it was considered that the ADR-induced cardiotoxicity contributed to toxic death and that DOC pre-administration decreased toxic death by relieving ADR-induced cardiotoxicity.

We thought that DOC pre-administration inhibited the cardiac muscle injury caused by ADR. However, the histopathology of the hearts of mice in the DOC-ADR and ADR groups showed mild cardiac muscle injury. Thus, the cardiotoxicity induced by ADR may exacerbate certain cardiac functions such as cardiac contraction and dilatation rather than induce myocardial tissue injury in this study.

Although the exact mechanisms underlying ADR-induced cardiac dysfunction are not fully understood, past reports have suggested many mechanisms such as the iron and free-radical hypothesis (16, 23, 24); the metabolite hypothesis (17, 25); the calcium overload hypothesis (26–28); the imbalance in myocardial electrolytes hypothesis (28); and the changes in adenylate cyclase (17, 25). In particular, there are two major hypotheses for the mechanism: the iron and free-radical hypothesis and the metabolite hypothesis (16). The iron and free-radical hypothesis states that ADR produces anthracycline semiquinone free radicals via NADPH-dependent reductase. In addition, iron (III) readily interacts with ADR, and this is followed by a redox reaction, wherein the iron atom accepts an electron, generating on iron (II) – ADR free radical complex. This radical complex can easily reduce oxygen, thereby leading to the generation of oxygen free radicals. These free radicals have the potential to cause damage to lipids and proteins (30). The metabolite hypothesis states that ADRol is nearly 30 times more potent than ADR at depressing contractility (17), and that ADRol inhibits the calcium pump of the sarcoplasmic reticulum, the Na⁺/K⁺ pump of the sarclemma, and the F0F1 proton pump of mitochondria. Thus, ADRol can affect myocardial energy metabolism, ionic gradients, and Ca²⁺ movement, ultimately impairing cardiac contraction and relaxation.

In this study, no differences were detected in plasma or heart ADR concentrations between the ADR and DOC-ADR groups. However, the plasma and heart ADRol concentrations were significantly different between the ADR and DOC-ADR groups at some sampling points. The metabolite hypothesis is that ADRol is more potent than ADR in inhibiting systolic and diastolic function, and there is a direct relationship between intracardiac ADRol accumulation and progressive impairment of both contractility and relaxation of the heart muscle (17, 25). The AUC of plasma ADRol and the cardiac ADRol concentration were higher in the DOC-ADR group, in which cardiotoxicity was significantly inhibited, than in the ADR group. Moreover, heart ADRol concentration was also higher in the DOC-ADR group than in the ADR group at all sampling points. Therefore, we considered that the pharmacokinetics of ADR do not affect the mechanism by which the pre-administration of DOC relieves ADR-induced cardiotoxicity.

The lipid peroxidation level in myocardial tissue in the DOC-ADR group was the same as that of the control group. However, the ADR group showed a significantly higher lipid peroxidation level than the control and DOC-ADR groups. Many previous studies have suggested that increased lipid peroxidation in myocardial tissue causes ADR-induced cardiac damage (31–33). It is reported that many free radical scavengers markedly inhibit the ADR-induced production of free radicals in myocardial tissue and that free radical scavengers prevent ADR-induced cardiotoxicity (31, 34). Therefore, we considered that DOC pre-administration inhibits ADR-induced cardiotoxicity by inhibiting ADR-induced production of free radicals in myocardial tissue and thereby improves the survival rate.

It is known that ADR-induced cardiotoxicity is inhibited by combining some free radical scavengers (34–37). However, DOC itself did not show radical scavenging ability (data not shown). Therefore, we consider that
DOC cannot directly prevent the ADR-induced cardiac injury. Since the DOC pre-administration protocol was the regimen to reduce only toxic death among several protocols in which DOC and ADR were given (38), DOC may induce the expression of biological factors that possess free radical scavenging ability for a few hours after its administration. In our previous study, we demonstrated using fluorogenic derivatization–liquid chromatography–tandem mass spectrometry that DOC pre-administration significantly increased the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) level of myocardial tissue compared with the ADR/DOC group, in which ADR and DOC were administered without any intervals between injections (39). Baek et al. proposed that GAPDH directly scavenges reactive oxygen species (ROS), based on the result that the overexpression of GAPDH in yeast cells resulted in an increased cellular antioxidative capacity (40). Furthermore, we suggested that the expression of a biological factor that participates in the production of free radicals is increased by DOC pre-administration using microarray analysis (data not shown). These changes may contribute to inhibiting the production of free radicals, and we are currently studying this matter. From these observations, we consider that DOC pre-administration decreases the amount of free radicals produced after ADR administration by inducing biological factors with radical scavenging ability.

Inhibiting ADR-induced cardiotoxicity is very important because ADR-induced cardiotoxicity is irreversible and fatal. DOC, an antitumor drug, relieves ADR-induced cardiotoxicity and augments the antitumor effects (9). Combination therapy involving ADR that not only inhibits ADR-induced cardiotoxicity but also augments the antitumor effects has been not reported in the past. Therefore, the combination of ADR and DOC is suggested to be very beneficial for cancer patients, providing a suitable dosing interval and dosing sequence are selected.

In conclusion, pre-treatment with DOC relieved ADR-induced cardiotoxicity. It was considered that the mechanism behind this involves the inhibition of the increase in myocardial tissue free radical production caused by ADR.

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