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Dosing time dependency of doxorubicin-induced cardiotoxicity and bone marrow toxicity in rats

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Abstract

Cardiac toxicity caused by doxorubicin (adriamycin) is a serious dose-limiting factor in the clinical situation. However, the influence of doxorubicin dosing time has not been clarified from the viewpoints of cardiotoxic development and its mechanism. In this study, we have investigated the dosing time dependency of doxorubicin-induced cardiotoxicity and bone marrow toxicity after repeated administration of doxorubicin in rats. When doxorubicin (5 mg kg^{-1}, i.p.) was administered every seven days (total of 30 mg kg^{-1}) at 3, 9, 15 or 21 h after the light was turned on (HALO), toxic death was significantly higher in the 9 HALO treated group than the other groups. When doxorubicin was injected every seven days for 28 days at 9 or 21 HALO, we measured the levels of creatine kinase, malondialdehyde (MDA; an index of lipid peroxide), and glutathione peroxidase (GPx) as markers of cardiotoxicity. On days 14 and 28, creatine kinase levels were significantly higher in the 9-HALO group compared with the 21-HALO group ($P<0.01$, respectively). On day 14, MDA levels increased significantly in the 9 HALO group compared with the 21 HALO group ($P<0.01$). A single dose of doxorubicin was administered at 9-h or 21-h after the light was turned on to investigate the dosing-time-dependent difference of the pharmacokinetics. The area under the plasma time–concentration curve showed a significant increase at 9 HALO compared with 21 HALO ($P<0.05$). These results suggested that the dosing-time-dependent difference of cardiotoxicity induced by doxorubicin was closely related to the daily variation of doxorubicin pharmacokinetics. In conclusion, the choice of optimal dosing time based on the chronopharmacokinetics of doxorubicin may decrease the cardiotoxicity and enable the practice of effective and safe chemotherapy of doxorubicin.

Introduction

To achieve safe chemotherapy it would be beneficial to relieve its adverse effects such as myelosuppression, vomiting and nausea. Many attempts have been made to decrease the adverse effects induced by antitumour drugs, and one such approach has been the chronopharmacological approach. It has been reported that many drugs have rhythm-dependent differences in their effects and pharmacokinetics (Ohdo et al 1997, 2001; Kobayashi et al 2000; To et al 2000). Chronotherapy is defined as the administration of medications to biological rhythms to optimize therapeutic outcomes and/or control adverse effects. These findings for antitumour drugs have been reported in man (von Roemeling & Hrushesky 1989; Lévi et al 1997; Thrall et al 2000; Kobayashi et al 2001).

Toxicity and efficacy of doxorubicin depends on dosing time in animals (Lévi et al 1980; Scheving et al 1980; Burns 1985). For concurrent administration of doxorubicin and cisplatin, it is reported that the chronotherapy of doxorubicin is useful in clinical studies (Hrushesky 1985; Barrett et al 1993). Cardiotoxicity must be given close attention during doxorubicin chemotherapy, because doxorubicin-induced myocardial damage is irreversible and can be lethal. The cardiotoxicity accumulates during the repeated administration of doxorubicin. In these studies, the influence of doxorubicin dosing time has not been clarified from the viewpoints of cardiotoxic development and its mechanism after the repeated administration of this agent, although doxorubicin-induced cardiotoxicity is an important issue.

In this study, we have investigated the dosing time dependency of doxorubicin-induced toxicity (toxic death, leucopenia and cardiotoxicity). Doxorubicin was administered every seven days in rats. Leucocyte counts, used as an index of leucopenia, were measured on day 3 after each doxorubicin administration. The cardiotoxicity was estimated using creatine kinase levels in serum (measured as a biomarker of damage to cardiac muscle), production of free radical formation (lipid peroxide) in cardiac tissue (one of most likely factors for cardiomyopathy induced by doxorubicin), and glutathione peroxidase (an antioxidant enzyme) activity in cardiac tissue. The dosing time dependency of doxorubicin pharmacokinetics was determined after a single dose.

Materials and Methods

Animals

Three-week-old male Donryu rats were purchased from Charles River Japan, Inc. (Yokohama, Japan). The animals were maintained two or three per cage for more than four weeks in two specific-pathogen free rooms, under a constant environment and a strictly controlled light condition (12-h light/dark). In room I, lights were turned on and off at 07 00 and 19 00 h, respectively, while in room II, lights were turned on and off at 19 00 and 07 00 h, respectively. During the acclimatization period food and water were freely available. The experiments were performed from May to August (1999 and 2000).

The Institutional Ethical Committee for Research on Animals granted approval for the experiments.

Preparation of doxorubicin

Doxorubicin (adriamycin) was supplied by Kyowa Hakko Kogyo Co. Ltd (Tokyo, Japan). The drug was dissolved in saline and prepared in a concentration of 1 mg/2 mL.

In a preliminary study, we looked for a dose of doxorubicin that would enable blood samples to be collected from all doxorubicin-treated groups from the tail vein for 28 days and the survival test (toxic death) could be finished within 56 days during which time doxorubicin would be administered every seven days. Doxorubicin at a dose of 2.5 mg kg^{-1} enabled collection of blood samples from the tail vein over 28 days in all doxorubicin-treated groups, and all rats survived for 56 days. Doxorubicin at a dose of 10 mg kg^{-1} enabled the recording of survival days within 56 days, but in many rats blood samples could not be collected for 28 days. However, 5 mg kg^{-1} doxorubicin satisfied the conditions fully, therefore this was the dose chosen for the study.

Circadian rhythm in tolerance (survival) of chronic doxorubicin administration

To study toxic death, doxorubicin (5.0 mg kg^{-1}) was intraperitoneally administered every seven days (total of 30 mg kg^{-1}) at 3, 9, 15, or 21 h after the light was turned on (HALO) (n = 10). Survival day was recorded over a 56-day period for each rat.

Doxorubicin dosing time-dependent myelosuppression

To estimate myelosuppression induced by doxorubicin administration, rats were divided into doxorubicin- and saline- (control) treated groups (n = 8 and 7, respectively, in each group). Doxorubicin (5.0 mg kg^{-1}) or saline was intraperitoneally administered 9 or 21 HALO every seven days for 28 days. Blood samples were temporally collected from the tail vein of each rat at 72 h (days 3, 10, 17 and 24) after each clock-time treatment with doxorubicin or saline and at the corresponding dosing time on day 0, and then leucocyte counts were measured. The change of leucocyte counts in the doxorubicin-treated groups was calculated as the percentage of leucocyte change in each rat from the initial value (day 0) at the corresponding dosing time.

Doxorubicin dosing-time-dependent cardiotoxicity

To estimate cardiotoxicity induced by doxorubicin administration, rats were divided into doxorubicin- and saline-(control) treated groups. Doxorubicin (5.0 mg kg^{-1}) or saline was intraperitoneally administered at 9 or 21 HALO every seven days for 28 days. On days 14 and 28 rats were killed at 9 or 21 HALO in the doxorubicin-treated and the control groups (n = 8 and 3–4, in each group, respectively), and the heart removed and immediately frozen at −80 °C until analysed. Blood samples were obtained from the inferior vena cava, and were quickly centrifuged at 3000 rev min^{-1} for 15 min. Plasma and serum were stored at −80 °C until analysed.

Chronopharmacokinetics of doxorubicin

Rats were divided into the 9- and 21-HALO-treated groups (n = 6). Blood samples were obtained from a tail vein at 2, 10, 30, 60, 180, 360 and 1440 min after doxorubicin (5.0 mg kg^{-1}, i.p.) administration. The samples were immediately centrifuged at 3000 rev min^{-1} for 15 min. Plasma was stored at −80 °C until analysed.

Measurement of cardiotoxicity

Lipid peroxide in the heart was assayed by determining the amount of thiobarbituric acid reactive substances (TBARs) using a modified thiobarbituric acid (TBA) method (Singal & Pierce 1986). Homogenates (10%) in hearts were prepared in 1.15% KCl. Homogenate (1 mL) was mixed with 50 μL 100 mM butylated hydroxytoluene and 1 mL 10% trichloroacetic acid. The solution was added to 0.5 mL 5 mM EDTA, 0.5 mL 8% SDS and 1.5 mL 0.6% thiobarbituric acid, and was incubated at 90 °C for 60 min. After cooling, 4 mL n-butanol was added to the solution and shaken for 1 min. The samples were centrifuged at 3000 rev min^{-1} for 15 min. The absorbance of the supernatant was detected spectrophotometrically at 535 and 520 nm and the difference in optical density between the two determinations was taken to be

the lipid peroxide level (nmol g^{-1}). The obtained absorbance was compared with that of malondialdehyde (MDA) as TBARs standard. The change in the rate of the MDA level in the doxorubicin-treated groups was calculated as the percentage of MDA change in each rat from the mean value in the control groups at the corresponding dosing time on days 14 and 28.

Glutathione peroxidase (GPx) activity in the heart was measured by following the decrease in absorbance of the reaction solution at 340 nm. Homogenates (10%) in hearts were prepared in 1.15 % KCl. The following solution was added to a 1-mL tube: 100 μL 1 M Tris-HCl (pH 8.0) containing 5 mM EDTA (pH 8.0), 20 μL 0.1 M glutathione, 100 μL 2 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH), 100 μL 10 U mL^{-1} glutathione reductase, 620 μL H_2O, and 50 μL cytosolic solution obtained after the homogenate was centrifuged at 20 000 *g* for 30 min. The tubes were incubated at 37 °C for 3 min. After incubation, the cytosolic solution and 10 μL 7 mM t-butyl hydroperoxide were added to a 1-mL cuvette, and the conversion of NADPH to nicotinamide adenine dinucleotide phosphate (NADP) was monitored by recording absorbance at 340 nm for 2 min. GPx activity was expressed as nmol NADPH oxidized to NADP min^{-1} (mg protein)$^{-1}$, with a molar extinction coefficient for NADPH at 340 nm of 6.22×10^6 (Paglia & Valentine 1967). The change in the rate of the GPx activity in the doxorubicin-treated groups was calculated as the percentage of the GPx change in each rat from the mean value in the control groups at the corresponding dosing time on days 14 and 28.

Creatine kinase in serum was measured using the CPK II-test Wako kit (Wako). The change in the rate of the creatine kinase level in the doxorubicin-treated groups was calculated as the percentage of creatine kinase change in each rat from the mean value in the control groups at the corresponding dosing time on days 14 and 28.

Measurement of doxorubicin plasma concentrations

Blood samples were obtained from a tail vein. The samples were immediately centrifuged at 3000 rev min^{-1} for 15 min. Plasma (0.1 mL) was mixed for 15 min with 0.1 mL solution buffer (0.01 M phosphate buffer (pH 3.0)–methanol (1:1)), 0.1 mL epirubicin (5 μg mL^{-1}) as the internal standard, 1 mL Colthoff buffer and 4 mL ethyl acetylate–n-propanol (4:1), and was centrifuged at 3500 rev min^{-1} for 15 min. The supernatants were dried with Speed Vac Plus SC110A (Savant Instruments, US) under reduced pressure. After drying completely, 300 μL solution buffer was added, mixed for 30 s, and sonicated for 5 min. The solutions were centrifuged at 3500 rev min^{-1} for 15 min. Supernatant (150 μL) was injected into the high-performance liquid chromatographic system that comprised a pump (LC-10AS, Shimadzu, Japan), a detector (RF-10A_{XL}, Shimadzu, Japan), and an analytical column (YMC-Pack ODS-AM, 150 × 4.6 mm i.d., YMC Co. Ltd, Japan). The mobile phase was H_2O–methanol–acetic acid (53:37:10), delivered at a flow-rate of 0.9 mL min^{-1}. The column effluent was monitored at ex 470 nm and em 585 nm.

Statistical analysis

The survival days were drawn with the Kaplan-Meier method and compared by the log-rank test. Statistical moment analysis was performed by calculating pharmacokinetic parameters such as area under the plasma time–concentration curve (AUC), mean residence time (MRT) and variance of residence time (VRT). The leucocyte counts on day 0 in the doxorubicin-treated groups, levels of creatine kinase, MDA and GPx in the control groups, and the doxorubicin concentration and pharmacokinetic parameters are shown as the mean ± standard deviation (s.d.). The other values are shown as the mean ± standard error (s.e.m.). Differences between two groups were analysed by Student's *t*-test. The Tukey-Kramer multiple comparisons test was used to assess the significance of differences among a few groups. A probability level of less than 0.05 was considered to be significant.

Results

Influence of dosing time on survival rate after repeated administration of doxorubicin

The deceased rats showed marked accumulation of ascites, and all rats treated at 9 HALO died within 50 days after the initiation of doxorubicin administration (Figure 1). The 9 HALO-treated group showed the worst survival rate of the doxorubicin-treated groups (compared with 3 and 15 HALO $P < 0.05$; compared with 21 HALO $P < 0.01$).

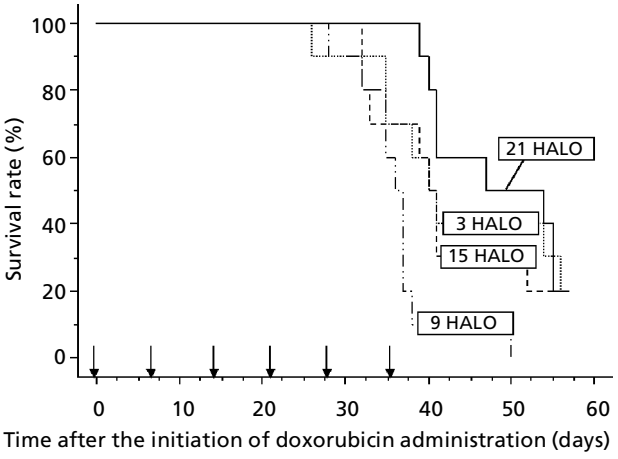

Figure 1 Dosing-time-dependent change in the survival rate after doxorubicin injection. Doxorubicin (5 mg kg^{-1}, i.p.) was administered every seven days (total of 30 mg kg^{-1}) at 3, 9, 15, or 21 h after the light was turned on (HALO) in rats (n = 10, respectively). The survival days were drawn with the Kaplan-Meier method. Mortality was significantly higher at 9 HALO compared with 3, 15 or 21 HALO (vs 3 and 15 HALO, $P < 0.05$; vs 21 HALO, $P < 0.01$).

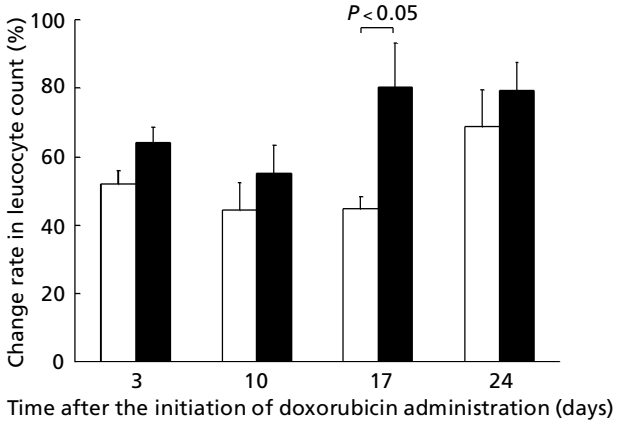

Figure 2 Dosing-time-dependent change of leucocyte count on days 3, 10, 17 and 24 after doxorubicin injection. Doxorubicin (5 mg kg^{-1}, i.p.) was administered every seven days for 28 days at 9 (open bar) or 21 h (closed bar) after the light was turned on (HALO) in rats (n = 8, respectively). The values shown are means ± s.e.m. The change in the rate of leucocyte counts in the doxorubicin-treated groups was calculated as the percentage of leucocyte change in each rat from the initial value (day 0) at the corresponding dosing time. The group treated at 9 HALO tended to decrease the leucocyte counts compared with that at 21 HALO, and the leucocyte count on day 17 was significantly lower at 9 HALO compared with 21 HALO ($P < 0.05$).

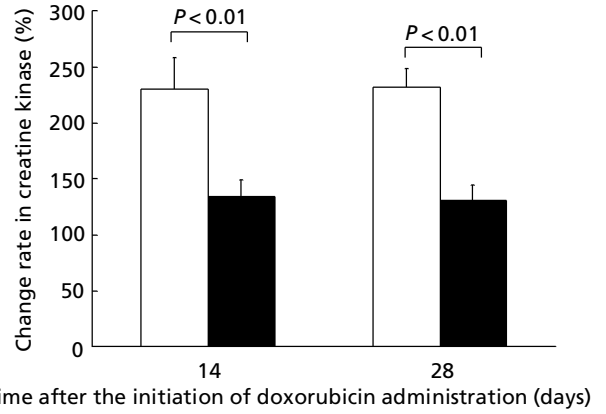

Figure 3 Dosing-time-dependent change in the rate of creatine kinase on days 14 and 28 after doxorubicin injection. Doxorubicin (5 mg kg^{-1}, i.p.) was administered every seven days for 28 days at 9 (open bar) or 21 (closed bar) h after the light was turned on (HALO) in rats (n = 8, respectively). The values shown are means ± s.e.m. The change in the rate of creatine kinase in doxorubicin-treated groups was calculated as the percentage of creatine kinase change in each rat from the mean value in the control group at the corresponding dosing time on days 14 and 28. On days 14 and 28, the change rate of creatine kinase in the 9 HALO treated group were significantly higher compared with those in the 21 HALO treated group ($P < 0.01$, respectively).

Influence of dosing time on change of leucocyte counts on days 3, 10, 17 and 24 after repeated administration of doxorubicin

Throughout this study, leucocyte counts in the control groups did not show significant differences on days 3, 10, 17 and 24 compared with day 0 at corresponding dosing time. Leucocyte counts in the doxorubicin-treated groups decreased significantly at corresponding dosing time on days 3, 10, 17 and 24 compared with those in the control groups ($P < 0.05$, respectively). Leucocyte counts on day 0 in the doxorubicin-treated groups were 11 031 ± 2147 and 7225 ± 1674 cells μL^{-1} (mean ± s.d.) at 9 and 21 HALO, respectively. These values showed a significant 24 h variation ($P < 0.01$). The initial values for leucocyte counts on day 0 varied markedly at the two different circadian times, and so the change rate in leucocyte counts in the doxorubicin-treated groups was shown as a function of the initial value and each value at corresponding dosing time on days 3, 10, 17 and 24 (Figure 2). Leucopenia was more severe in the group treated at 9 HALO compared with 21 HALO. On day 17, the change rate in leucocyte counts was significantly lower at 9 HALO compared with 21 HALO ($P < 0.05$).

Influence of dosing time on cardiotoxicity after repeated administration of doxorubicin

Creatine kinase levels in the control groups were 45.7 ± 6.8 and 50.1 ± 10.1 IU L^{-1} (mean ± s.d.) at 9 and 21 HALO on day 14 and 40.4 ± 5.3 and 49.4 ± 13.1 IU L^{-1} at 9 and 21 HALO on day 28. These values did not show a significant difference. At 9 HALO, the creatine kinase levels on day 14 and 28 increased significantly in the doxorubicin-treated groups compared with the control group ($P < 0.05$, respectively), but were kept normal in those treated at 21 HALO. The change in the rate of creatine kinase was shown as a function of the mean value in the control groups and each value in the doxorubicin-treated groups at corresponding dosing time on days 14 and 28 (Figure 3). The change in the rate of creatine kinase levels on days 14 and 28 was significantly higher in the 9 HALO treated group compared with the 21 HALO treated group ($P < 0.01$, respectively).

The MDA level in the control groups was 8.8 ± 1.8 and 11.6 ± 1.3 IU L^{-1} (mean ± s.d.) at 9 and 21 HALO, respectively, on day 14 and 10.6 ± 1.9 and 12.9 ± 2.2 IU L^{-1} at 9 and 21 HALO, respectively, on day 28. These values showed a significant 24 h variation on day 14 ($P < 0.05$). The change in the rate of MDA was shown as a function of the mean value in the control groups and each value in the doxorubicin-treated groups at corresponding dosing time on days 14 and 28 (Figure 4). The change rate in MDA levels was higher in the 9 HALO group compared with the 21 HALO group and it showed a significant dosing time-dependent difference on day 14 ($P < 0.01$).

GPx activity in the control groups was 50.9 ± 16.2 and 48.1 ± 6.3 IU L^{-1} (mean ± s.d.) at 9 and 21 HALO, respectively, on day 14 and 48.1 ± 8.9 and 55.0 ± 5.9 IU L^{-1} at 9 and 21 HALO, respectively, on day 28. These values did not show a significant difference. The change in the rate of

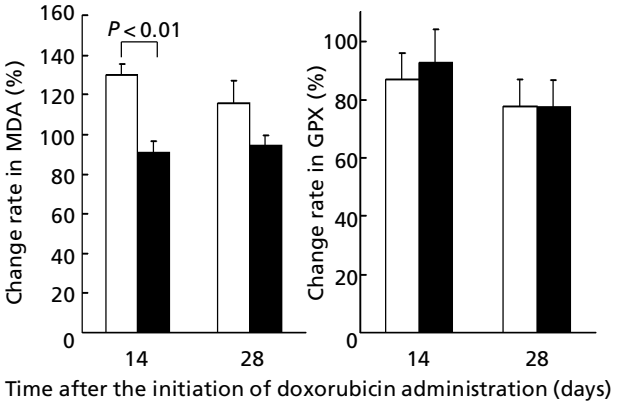

Figure 4 Dosing-time-dependent change in the rate of MDA (left panel) as an index of lipid peroxide itself, and GPx (right panel) on days 14 and 28 after doxorubicin injection. Doxorubicin (5 mg kg^{-1}, i.p.) was administered every seven days for 28 days at 9 (open bar) or 21 (closed bar) h after the light was turned on (HALO) in rats (n = 8, respectively). The values shown are means ± s.e.m. The change in the rate of MDA or GPx in the doxorubicin-treated groups was calculated as the percentage of MDA or GPx change in each rat from the mean value in the control group at the corresponding dosing time on days 14 and 28, respectively. On days 14 and 28, the change rate of MDA was higher in the 9 HALO group compared with the 21 HALO group (day 14, $P < 0.01$). The change rate of GPx activity did not show a significant difference in the 9 HALO and 21 HALO groups.

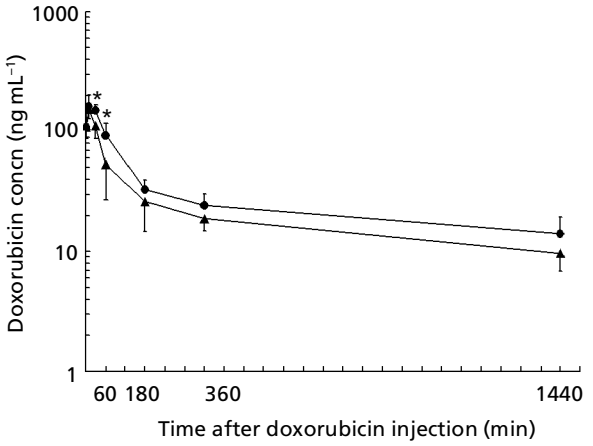

Figure 5 Plasma concentration of doxorubicin after drug (5 mg kg^{-1}, i.p.) administration at 9 (●) or 21 (▲) h after the light was turned on (HALO) in rats (n = 6, respectively). The values shown are means ± s.d. The plasma doxorubicin concentrations at 30 and 60 min after doxorubicin injection are significantly higher in the doxorubicin-treated group at 9 HALO compared with the 21 HALO treated group ($P < 0.05$, respectively).

GPx was shown as a function of the mean value in the control groups and each value in the doxorubicin-treated groups at corresponding dosing time on days 14 and 28 (Figure 4). The change rate in GPx activity on day 28 in the groups treated at 9 HALO and 21 HALO decreased to approximately 77% of the control group.

Chronopharmacokinetics of doxorubicin concentrations

Plasma doxorubicin concentrations in the doxorubicin-treated group were higher at 9 HALO than 21 HALO (30 and 60 min, $P < 0.05$, respectively; Figure 5). The AUC showed a significant increase in the 9 HALO group compared with the 21 HALO group ($P < 0.05$; Table 1). MRT and VRT showed no significant dosing-time-dependent difference.

Discussion

Doxorubicin-induced cardiotoxicity, including congestive heart failure, was reported after a single administration of a high dose (acute toxicity) and repetitive administration of low doses (chronic toxicity) in several animal species (Rosenoff et al 1975; Jensen et al 1984; Al-Harbi et al 1992). The toxicity depended on the dosing schedule and total dose, and the cumulative dosage of 15–20 mg kg^{-1} produced high mortality, accumulation of ascites, decrease in body weight gain and depressed cardiac function in rats (Olson & Capen 1978; Iliskovic & Singal 1997). In this study, toxic death was observed in doxorubicin-treated groups when 20 mg kg^{-1} was used as the total dose of doxorubicin. Death varied significantly depending on dosing time despite the same dosage in all groups, and accumulation of many ascites was shown in the deceased rats. On day 28, rats were killed after doxorubicin (5.0 mg kg^{-1}) was administered four times every seven days (total dose: 20 mg kg^{-1}). Ascites, one of the indicators of congestive heart failure caused by doxorubicin,

Table 1 Influence of dosing time on plasma pharmacokinetic parameters of doxorubicin.

Pharmacokinetic parameters	Time of drug injection		Statistical significance
	9 HALO[a]	21 HALO[a]	
$AUC_{0-1440\,min}$ (ng × min ml^{-1})	41223.8 ± 7101.0	30438.7 ± 7238.7	$P < 0.05$
$MRT_{0-1440\,min}$ (min)	425.8 ± 67.4	420.0 ± 35.5	NS
$VRT_{0-1440\,min}$ (min^2)	238535.5 ± 33234.6	230942.0 ± 21896.0	NS

[a] HALO is *h*ours *a*fter the *l*ight was turned *o*n. Data are the mean ± s.d. of 6 rats.

was shown in all rats of the 9 HALO-treated group (ascites: 5.9 mL (median)), although the 21 HALO-treated group did not produce ascites in three of eight rats (ascites: 2.4 mL (median)). We focused on two dosing time points (9 and 21 HALO) in which the mortality was the highest and lowest and investigated a dosing time dependency of doxorubicin-induced cardiotoxicity and its mechanism.

Creatine kinase is released from cardiac muscle into blood by cardiotoxicity of doxorubicin (Robison et al 1989) and is measured as a biomarker of damage to cardiac muscle in many studies (Kojima et al 1993; Behnia & Boroujerdi 1999). After a single high dose of doxorubicin (15–20 mg kg^{-1}) in rats the creatine kinase level increased temporarily and recovered normal values at approximately seven days (Al-Harbi et al 1992; Kojima et al 1993). When doxorubicin (2 mg kg^{-1}) was administered every seven days for 12 weeks in rats, creatine kinase levels were significantly elevated at eight weeks compared with pretreatment (total dose: 16 mg kg^{-1}) (Olson & Capen 1978). In this study, although creatine kinase levels on days 14 and 28 were kept normal during the active phase (21 HALO) after doxorubicin was administered every seven days for 28 days, creatine kinase levels during the rest phase (9 HALO) were twice as high compared with the control group. The change in the rate of the creatine kinase level during the rest phase was significantly greater than that during the active phase. Thus, doxorubicin-induced cardiotoxicity showed a significant dosing-time-dependent difference.

Though the cardiotoxic mechanism induced by doxorubicin has not been completely clarified, composite factors may contribute to the cardiotoxicity (Revis & Marusic 1978; Singal & Pierce 1986; Tong et al 1991). When doxorubicin was repeatedly administered, lipid peroxide levels, which were measured as MDA levels in this study, increased and GPx activity decreased in cardiac tissue (Siveski-Iliskovic et al 1994; Iliskovic & Singal 1997; Matsui et al 1999). In this study, the mechanisms underlying the dosing time dependency of cardiotoxicity induced by doxorubicin were investigated using lipid peroxide, which is a causal compound of cardiac injury (Myers et al 1977), and GPx, which is a myocardial antioxidant enzyme (Revis & Marusic 1978), and is the primary enzymatic mechanism for the disposal of lipid peroxide induced by doxorubicin in several antioxidant enzymes (Li et al 2000). The lipid peroxide levels in cardiac tissue during repeated administration of doxorubicin were markedly higher during the rest phase than the active phase. GPx activity decreased in both dosing time groups compared with the control groups and showed no significant dosing-time-dependent difference. Although the generation of lipid peroxide depended on dosing time, there was no dosing-time-dependent change of GPx activity. Thus, the process of generation rather than disposal of lipid peroxide may contribute much to the 24-h variation of lipid peroxide. Doxorubicin-promoted free radicals such as lipid peroxide were suggested to play an important role in the cardiotoxicity (Doroshow 1983; Kaul 1993), and to cause heart dysfunction (Gupta & Singal 1989). As lipid peroxide levels increased in cardiac tissue, creatine kinase levels increased in blood. Moreover, lipid peroxide depended significantly on dosing time, and the dosing time dependency of lipid peroxide corresponded with that of creatine kinase. It might be suggested that myocardial injury resulted from the accumulation of lipid peroxide during the repeated administration of doxorubicin.

The AUC of doxorubicin was significantly higher during the rest phase than the active phase. The mortality, lipid peroxide and creatine kinase levels showed high values when the AUC of doxorubicin was high, and low values when the AUC of doxorubicin was low. The dosing-time-dependent difference of doxorubicin pharmacokinetics appeared to contribute to that of doxorubicin chronotoxicity. The cytotoxic effect of doxorubicin depends on the doxorubicin concentration (AUC) in cells and blood (Simoyama 1975). Chronopharmacokinetics of doxorubicin were reported in 18 patients treated concomitantly with 5-fluorouracil, doxorubicin and cyclophosphamide in the morning or evening (Canal et al 1991). The clearance of doxorubicin decreased significantly during the rest phase compared with the active phase, resulting in a longer elimination half-life and an increase in the AUC. The hepatic blood flow showed a significant 24-h variation with a peak in the early morning and a trough in the early evening in healthy subjects (Lemmer & Nold 1991). The 24-h variation of hepatic blood flow was related to that of doxorubicin clearance in breast cancer patients (Canal et al 1991). The hepatic blood flow in rats showed a significant 24-h variation with a peak in the active phase and a trough in the rest phase (Labrecque et al 1988). Namely, the AUC of doxorubicin in rats and in man increased during the rest phase showing lower hepatic blood flow compared with the active phase showing higher hepatic blood flow. Furthermore, the activity of many reductases showed 24-h variation with maximum levels during the active phase (North et al 1981; Hwa et al 1992). Although the 24-h variation of aldo-keto reductase, which is the main metabolic enzyme of doxorubicin, was not clarified, the aldo-keto reductase in addition to these reductases may also have high activity during the active phase. We hypothesized that the 24-h variation of aldo-keto reductase contributed to the dosing time-dependent difference in doxorubicin toxicity. From these findings, the 24-h variation of the functions of metabolism and excretion such as hepatic blood flow and aldo-keto reductase appeared to cause the dosing-time-dependent AUC of doxorubicin.

It was reported that doxorubicin stimulated mitochondrial superoxide formation in a dose-dependent manner in-vitro (Doroshow 1983). In this study, the lipid peroxide levels showed high values when the AUC of doxorubicin was high, and low values when the AUC of doxorubicin was low. These results suggested that the 24-h variation of doxorubicin pharmacokinetics contributed to that of lipid peroxide production. Moreover, the creatine kinase levels showed high values when lipid peroxide levels were high, and low values when lipid peroxide levels were low. The findings of this study suggested that the 24-h variation of cardiotoxicity induced by doxorubicin was related to that of doxorubicin pharmacokinetics.

In this study, we showed that the toxicity caused by doxorubicin depended on the 24-h variation of pharmacokinetics. From these results, reducing the doxorubicin dose may be considered as one of the means to decrease toxicity. However, the chronotoxicity was not satisfactorily investigated from a viewpoint of sensitivity in an organism. Generally, the 24-h variations of adverse effect and antitumour effect often show the different pattern of rhythmicity associated with the pharmacokinetics of drugs and the sensitivities in an organism to the drugs. Therefore the dosing time to maximize the antitumour effect does not necessarily coincide with the dosing time to minimize the adverse effect. Since the various rhythmic factors described above influenced the effect of the drug, the adjustment of dosage neglecting these background factors should not be performed simply to decrease the toxicity and increase the antitumour effect. Thus, it will be necessary to select the optimal dosing schedule such as adjustment of dosage and dosing time associated with not only the 24-h variation of pharmacokinetics but also that of sensitivity in normal and tumour cells.

In conclusion, the cardiotoxicity caused by doxorubicin depended on the dosing time. These daily variations corresponded with 24-h variation of pharmacokinetics of doxorubicin. The dosing time dependency of pharmacokinetics as one of the mechanisms in doxorubicin-induced cardiotoxicity may be an important factor. Therefore, choosing the optimal dosing time associated with the chronopharmacokinetics of doxorubicin may lead to safer chemotherapy with doxorubicin.

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