and urine within 20 min to 3 h in pigs fed 3-ADON, whereas 3-ADON is not detectable in plasma and urine (Eriksen et al., 2003) indicating that 3-ADON is likely deacetylated to DON after feeding. Our laboratory previously found that 15-ADON and DON were similar in their ability to induce feed refusal and growth retardation in mice (Forsell et al., 1987). While it is generally assumed that, like 3-ADON, 15-ADON is deacetylated in the gut, no data exist yet on the toxicokinetics of 15-ADON.

FX and NIV induced remarkably more robust and persistent feed refusal than did DON or the two ADONs, particularly at higher doses. Interestingly, the anorectic potencies of FX and NIV were reversed when comparing IP and oral exposure. Consistent with these findings, Ueno et al. (1971) observed that in mice the oral LD50 of NIV is 38.9 mg/kg, whereas for FX it is 4.5 mg/kg. Likewise in rats, the oral LD50s of NIV and FX are 19.5 and 4.4 mg/kg, respectively (Kawasaki et al., 1990; Ueno et al., 1971). Poapolathep et al. (2003) determined that the increased oral potency of FX is due to its more efficient absorption (5- to 10-fold) in the gastrointestinal tract than NIV in rodents. Thus when mice are given NIV by oral administration, a large proportion of the toxin might pass through the gastrointestinal lumen and remain on the mucosal surface of the gastrointestinal tract without being absorbed. Interestingly, once absorbed, FX is metabolized to NIV in the liver and kidney suggesting that the latter congener mediates toxicity in vivo.

The mechanisms by which DON and other 8-ketotrichothecenes cause anorexia are complex, but likely to involve both neuroendocrine hormones and cytokines. DON induces c-Fos in the circum ventricular organs of the brain and surrounding structures suggesting that the toxin might directly target the central nervous system (CNS) and modulate anorectic neurocircuitry, eventually causing anorexia due to satiety or sickness behavior (Girardet et al., 2011a,b). Consistent with direct action on the central nervous system, we previously have determined that DON distributes to the brain rapidly after oral exposure (Pestka et al., 2008).

Recently, Girardet et al. (2011b) have elegantly demonstrated that DON activates central anorectic pathways in mice by stimulating pro-opiomelanocortin (POMC) and nesfatin-1 expressing neurons in key hypothalamic nuclei and nucleus tractus solitarius (NTS) (Girardet et al., 2011b). POMC activation is related to food intake reduction and increased energy expenditure (Berthoud, 2002; Schwartz et al., 2000), while nesfatin-1 is evokes reduced food intake (Oh et al., 2006). Thus, expression and release of these hormones in the brain likely participate in DON-induced anorexia. In analogous fashion, subsequent increases in food in take might similarly be due to changes in expression of orexigenic peptides in the hypothalamus (Kobayashi Hattori et al. 2011; Vuagnat et al., 2000). It might be further speculated that the mechanisms by which the 3-ADON, 15-ADON, FX and NIV induce anorexia are the same as DON, however, further research will be needed to clarify this possibility.

DON is well-known to rapidly activate the innate immune response and evoke increased expression release of proinflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6(IL-6) and tumor necrosis factor-α (TNF-α) (Pestka, 2010a). These proinflammatory cytokines cause anorexia following either peripheral or central injection (Kelley et al., 2003; Plata-Salaman, 1998; Plata-Salamán et al., 1996; Sonti et al., 1996). Interestingly, DON upregulates expression of these cytokines not only in peripheral organs such as spleen, liver, lung and kidney (Amuzie et al., 2008; Amuzie et al., 2009; Pestka and Amuzie, 2008) but also centrally in the hypothalamus and dorsal vagal complex which are associated with regulating appetite (Girardet et al., 2011a). Therefore, DON-induced anorexia might also be mediated in part by cytokines in the peripheral and central system.

Taken together, the data presented herein indicated that 3-ADON, 15-ADON, FX and NIV dose-dependently caused anorexia in mice. Measurement of the anorectic potencies of these congeners is valuable because they predict the potentials for both (1) food refusal and sickness responses observed in animals and possibly humans following acute exposure and (2) growth retardation resulting from reduced food intake during chronic exposure. These data could thus be used in the future to establish toxic equivalency factors applicable to risk assessment. The NOAELs and LOAELs for the four 8-ketotrichothecenes determined here and for DON previously (Flannery et al., 2011), summarized in Table 1, indicate that the anorectic potencies of 8-ketotrichothecenes generally follow rank orders of NIV > FX > DON ≈ 3-ADON ≈ 15-ADON for IP exposure and FX > NIV > DON ≈ 3-ADON ≈ 15-ADONfor oral exposure. Both 3-ADON and 15-ADON induced rapid and transient anorectic responses similarly to that of DON, whereas FX and NIV evoked a more persistent anorexia, particularly at higher doses. All four toxins shared with DON, the capacity to provoke stronger anorexic effects following IP exposure as compared to oral exposure which likely reflect different bio availabilities following exposures to these toxins by the two routes. Future research efforts should be directed towards understanding mechanisms for 8-ketotrichothecene-induced anorexia, particularly in regard to the contribution of neuroendocrine hormones and cytokines.

Table 1
Comparison of anorectic effects of the 8-ketotrichothecenes.

Toxin (mg/kg bw)	IP		Oral	
	NOAEL[b]	LOAEL[c]	NOAEL[b]	LOAEL[c]
DON[a]	0.5	1	1	2.5
3-ADON	0.5	1	1	2.5
15-ADON	0.5	1	1	2.5
FX	0.025	0.25	0.025	0.25
NIV	0.01	0.1	0.1	1

[a] Data for DON were from Flannery et al. (2011).
[b] NOAEL = no observed adverse effect level.
[c] LOAEL = lowest observed adverse effect level.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Letter

Arrhythmias and alterations in autonomic nervous function induced by deoxynivalenol (DON) in unrestrained rats

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ABSTRACT — This study aimed to determine the cardiac and autonomic nervous effects of deoxynivalenol (DON), a representative mycotoxin which frequently contaminates cereal grains, in conscious rats that had been implanted with telemetric transmitters. Four groups of rats given subcutaneous injections of 0.5, 1.0, or 2.0 mg/kg of DON or propylene glycol (vehicle solution) were used in the experiments. Telemetric electrocardiogram (ECG) recordings were performed for 2 weeks or longer during the pre- and post-DON injection period. The present study demonstrated that DON caused marked arrhythmias, such as second-degree atrioventricular block, atrial bradycardia, supraventricular extrasystole, and ventricular extrasystole, at 3 hr or later (mostly at 10-20 hr) after the DON-injection, which were accompanied by a significant increase in heart rate (HR) and a significant decrease in total power and low- and high-frequency power during the period from 90 to 180 min after the injection. In conclusion, it was elucidated that DON produces significant cardiac dysfunction and transient inhibition of the autonomic nervous function in conscious rats at a dose of 0.5 mg/kg s.c. or more.

Key words: Arrhythmia, DON, ECG, Mycotoxin, Telemetry

INTRODUCTION

Deoxynivalenol (DON), "vomitoxin", is a representative of the trichothecene group (type B) of mycotoxins produced by *Fusarium*, and it contaminates cereal grains, such as wheat, barley, maize, and flour (Pestka and Smolinski, 2005; Pestka, 2008). The different groups of trichothecene contain T-2 and HT-2 toxins (type A). Many studies have reported that DON intake induces significant health effects, including anorexia, reduced weight gain, and immunotoxicity in humans and experimental animals (Pestka and Smolinski, 2005). The symptoms of DON toxicity in rats have been reported to include gastrointestinal hemorrhaging, hematuria, death at doses ranging from 0.1 to 104 mg/kg (Bosch *et al.*, 1989), and reduced fertility (Morrissey and Vesonder, 1985), and those in mice include immune suppression at a high dose, 10 ppm (diet) or 0.75 mg/kg (gavage) (Bondy and Pestka, 2000), or immunostimulatory effects, such as autoimmune-like disorders, e.g., IgA nephropathy (Rotter *et al.*, 1996).

On the other hand, several studies have indicated that T-2 toxin, which is another of the trichothecenes produced by *Fusarium*, induces functional and morphological alterations in the cardiovascular system in experimental animals, and significant decreases in systemic blood pressure and systolic left ventricular pressure in rats following the subcutaneous injection of 1 or 2 mg/kg of T-2 toxin (Magnuson *et al.*, 1987), and an increase in systemic blood pressure and arrhythmia lasting for 6 to 8 hr was observed in rats i.v. administered T-2 toxin at doses ranging from 0.5-2 mg/kg (Feuerstein *et al.*, 1985). In addition, it has been reported that all cultured myocytes ceased beating at 10 to 30 min after T-2 toxin application at concentrations of 250 μg/ml or higher (Yarom *et al.*, 1986).

These findings suggest that some trichothecenes possess potent cardiac toxicity. However, there is little evidence about the cardiac effects of DON, although cardiac lesions combined with calcified pericarditis were caused by the ingestion of a diet containing 10 to 20 ppm of DON for a few weeks (Robbana-Barnat *et al.*, 1987). Therefore, it is necessary to clarify whether DON has

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hazardous effects on cardiac function through a detailed investigation.

The aim of this study was to evaluate the effect of DON on cardiac and autonomic nervous functions in unrestrained rats using a telemetry recording system. These data should provide valuable knowledge about pathophysiological toxicity in human and non-primate animals affected by mycotoxicosis due to trichothecenes.

MATERIALS AND METHODS

Animals

The experiment was performed using 24 male Wistar rats which were purchased from Japan SLC, Inc. (Shizuoka, Japan) at 8 weeks of old and having the body weight of 230-250 g at 10 weeks of old when the telemetric device was implanted. Each rat was maintained with *ad libitum* access to food and water in an individual cage within an isolation chamber maintained under controlled lighting (light-dark cycle, light = 12:00-24:00, dark = 24:00-12:00) and temperature conditions (24°C). All rats were fully adapted to these breeding environments during experiments.

Implantation of telemetry device

Each rat underwent one-week adaptation period before the surgical operation. Then, a small telemetry device (weight = 3.9 g, volume = 1.9 cc; TA10ETA-F20, Data Sciences International, St. Paul, MN, USA) for transmitting electrocardiogram (ECG) was implanted into the dorsal subcutaneous region under systemic anesthesia with 30 mg/kg (i.p.) administered pentobarbital sodium. Paired wire electrodes that came with the telemetry device were placed under the skin of the dorsal and ventral thorax to record the apex-base (A-B) lead ECG.

Telemetric measurements of ECG and heart rate variability (HRV)

ECG telemetry recording systems provide useful information on cardiac function in unrestrained animals, including information about heart rate (HR), heart rhythm, and abnormal ECG waveforms. In addition, autonomic nervous function can be evaluated using this measuring system, in which the power spectrum is obtained by Fast Fourier Transform (FFT) analysis of the frequency component of the R-R interval on ECG in which the basic methodology is based on the Cooley-Tukey FFT algorithm (Cooley and Tukey, 1965). In rats, as in humans, two major spectral components exist, the low frequency (LF) (0.1-1.0 Hz) and high frequency (HF) (1.0-3.0 Hz) components, as shown in Fig. 1. Studies using autonomic nervous blockades have indicated that the LF component is influenced by both sympathetic and parasympathetic nervous activity and that the HF component is only affected by parasympathetic nervous activity. Accordingly, the LF/HF ratio indicates the balance between sympathetic and parasympathetic nervous activity (Kuwahara *et al.*, 1994).

Injection protocol

All rats were randomly divided into four DON or vehicle injection groups (n = 6/group). The DON was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan) and dissolved in 1 ml of propylene glycol. Then, 0.5, 1.0, or 2.0 mg/kg of DON with each volume of 0.7 ml/kg was subcutaneously injected into the rats with 11 weeks of old. Another group of rats was subcutaneously injected with 0.2 ml of propylene glycol without DON, which served as a vehicle control. All injections were performed at 12:00 when the light period was started on each injection day. This injection time was selected to avoid the large disturbance to biorhythm by handling stress to rats due to injections in mid-time during the light or dark period. In the present study, the administration route of DON was selected as the subcutaneous injection, but not as oral administration, since the trichothecene group of mycotoxin has usually strong gastrointestinal inflammation, anorexia and in some animal species vomit,

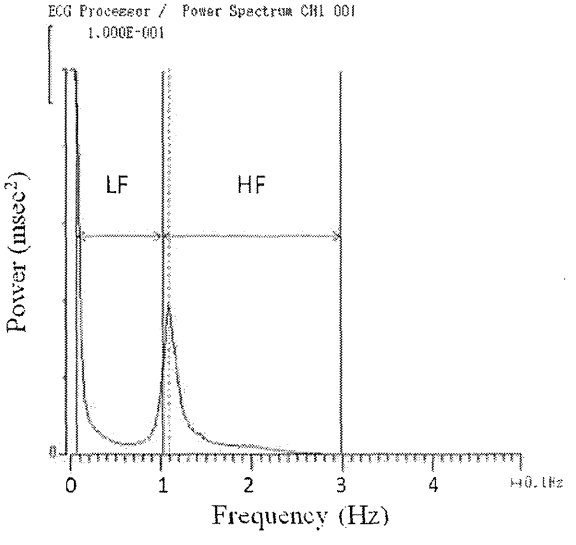

Fig. 1. An example of the mean power spectrum of the RR interval. The power spectrum was obtained for 30 min from a rat in the control group.

masking the toxic nature of the mycotoxin to the extra-digestive organs. The doses of DON injected in the present study was chosen by referring to the past reports on the cardiac effects by T-2 toxin (Feuerstein *et al.*, 1985; Magnuson, *et al.*, 1987).

ECG signal acquisition and data analysis

One week after the surgery for implantation of telemetry device, ECG signals were recorded from each rat in a cage that had been placed on a signal-receiving board (RA 1610, Data Sciences International). The ECG data sampled continuously at 1 msec intervals and all data analysis, including FFT analysis and ECG-wave components, were performed using an ECG processor analyzing system (SRV2W, Softron, Tokyo, Japan) equipped on a personal computer in series with an analog-digital converter, and the ECG data were stored on an external hard disk.

The ECG-wave components, i.e., PR interval, QRS duration, QT interval, and the autonomic nervous activity (HRV), as well as HR, were analyzed at 30 min intervals before and after the DON or vehicle injection. Furthermore, the ECG waveform and heart rhythm (R-R interval) were automatically or manually evaluated in order to detect episodes of arrhythmia before and after the DON administration.

Statistical analysis

The results on HR and HRV values among all groups before and after vehicle- or DON-injection were statistically evaluated by Two-way repeated-measures analysis of variance (Two-way repeated-measures ANOVA). Moreover, Kruskal-Wallis one-way analysis of variance (Shirley-William test) was used to test significant differences between control and treatment groups for PR interval, ORS duration, QT interval, HR and HRV values. The occurrence of arrhythmia was evaluated by analysis of variance measuring the frequency of episodes of arrhythmia. The data showing P values less than 0.05 were regarded as significant difference.

All experiments were conducted in accordance with the Animal Experimentation Guidelines of the University of Tokyo and approved by the institutional Animal Care and Use Committee of the Graduate School of Agricultural and Life Sciences at the University of Tokyo.

RESULTS

HR

The representative changes in HR that occurred during the telemetric ECG recording period are shown in Fig. 2, and periodic alterations in HR that were dependent on the light-dark cycle were revealed. Short-term changes in HR were observed before and immediately after the DON-injection, as shown in Fig. 3. Significant differences ($P < 0.05$, Two-way repeated-measures ANOVA) were observed for both time and doses of DON injected. There were significant increases in HR in the 1.0 and 2.0 mg/kg-DON groups ($P < 0.05$) from 90 to 150 min, while at 180 min, only the 2 mg/kg-DON group showed a significant difference ($P < 0.05$). At 0, 30, or 60 min after DON administration, no significant differences were recognized between the control group and the DON-injected groups. Also, there was no significant difference in the 0.5 mg/kg-DON group at any time point.

PR interval

The changes in the PR interval observed after the administration with vehicle or DON (0.5-2.0 mg/kg) are shown in Table 1. No significant difference was observed in the 0.5 or 1.0 mg/kg-DON groups compared with the control. However, in the 2.0 mg/kg-DON group, a significant increase ($P < 0.05$) in the PR interval compared with that in the control group was detected at 120 min.

QRS duration

The changes in the QRS duration that occurred after vehicle- or DON-administration are shown in Table 2. No significant difference was present in any treatment group compared with the control group.

QT interval

The changes in the QT interval observed after DON-administration are shown in Table 3. There were no QT interval differences in the 0.5 mg/kg-DON or 1 mg/kg-DON group compared with the control. However, in the 2.0 mg/kg-DON group, the QT interval was significantly increased at 60 min after the DON injection ($P < 0.05$).

Arrhythmia occurrence

DON administration at all doses clearly induced arrhythmia, as represented by second-degree atrioventricular (AV) block (typically following first-degree AV block), ventricular extrasystole, supraventricular extrasystole, nodal escaped beat (parasystole), and atrial bradycardia (Fig. 4). The occurrence of arrhythmia after the DON injection is summarized in Table 4. The frequency of second-degree AV block episodes increased significantly in a dose-dependent manner (ANOVA, $P < 0.05$). These arrhythmia episodes usually lasted for several seconds. A relatively large number of ventricular extrasystole episodes (premature ventricular contraction) were observed without a dose-dependent manner, even at the lowest

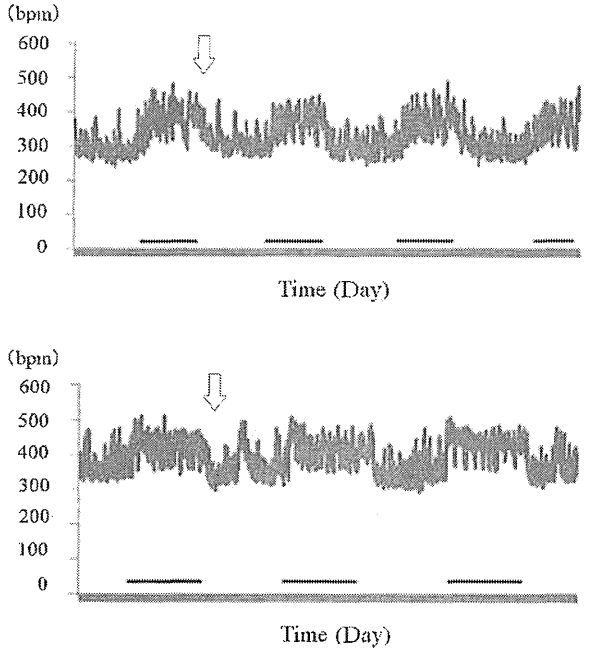

Fig. 2. Representative changes in heart rate by recording telemetric ECG in rats. The vehicle solution in A (upper panel; control group) and 1.0 mg/kg of DON in B (lower panel; DON-group) was subcutaneously injected at the point indicated by the arrow. The underlined section represents the dark period.

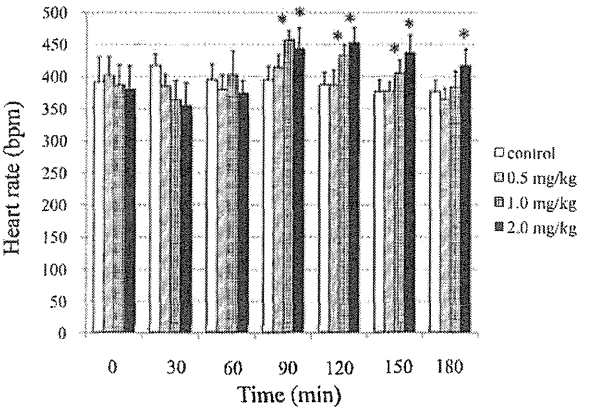

Fig. 3. Changes in heart rate in response to DON-injection. The asterisks show significant differences at $P < 0.05$ (*) from the control value at each time point. Zero min is the time before the vehicle- or DON-injection. Data are expressed as the mean ± S.D.

dose of DON (0.5 mg/kg, s.c.). These arrhythmia episodes appeared after 3 hr, mostly at 10-20 hr, following the DON administration. However, no obvious arrhythmias including ventricular extrasystole were observed in the control group.

HF power

The changes in HF power observed after vehicle or DON administration are shown in Fig. 5. Significant differences ($P < 0.05$, Two-way repeated-measures ANOVA) were observed for both time and doses of DON injected. Significant decreases ($P < 0.05$) were recognized at 90 min in the 0.5 mg/kg-DON group, at 90 min and 150 min in the 1.0 mg/kg-DON group, and at 90, 120, 150 and 180 min in 2.0 mg/kg-DON group if compared with control group at each time point.

LF power

The changes in LF power that occurred after vehicle or DON administration are shown in Fig. 6. Significant differences ($P < 0.05$, Two-way repeated-measures ANOVA) were found for both time and doses of DON injected. Significant differences ($P < 0.05$) were observed at 90 min in the 0.5 mg/kg-DON group, at 90 min and 120 min in the 1 mg/kg-DON group, and in the 2.0 mg/kg-DON group at all time-points after 90 min if compared with control group at each time point. The extent of decrease of LF power seemed to be most potent at 90 min.

LF/HF ratio

The changes in the LF/HF ratio after vehicle or DON administration are shown in Fig. 7. Significant differences ($P < 0.05$, Two-way repeated-measures ANOVA) were found for both time and doses of DON injected. At 90 min after DON injection, all DON groups showed a significant decrease ($P < 0.05$) in the LF/HF ratio compared with that in the control group. This significant decrease was also recognized at 120 min in the 2.0 mg/kg-DON group ($P < 0.05$).

Total power

The changes in total power that occurred after vehicle or DON administration are shown in Fig. 8. Significant differences ($P < 0.05$, Two-way repeated-measures ANOVA) were found for both time and doses of DON injected. The total power was significantly increased at 30 min after the DON injection in all DON groups, while significant decrease ($P < 0.05$) was recognized from 90 to 150 min in 1.0 mg/kg- and 2.0 mg/kg-DON groups and also at 180 min 2.0 mg/kg-DON group compared with that in the control.

Table 1. PR intervals in the control and DON-groups

	Time (min) after administration with vehicle (control) or DON (0.5-2.0 mg/kg)					
	30	60	90	120	150	180
Control	43.76 ± 1.42	43.33 ± 0.82	42.61 ± 1.19	42.15 ± 0.82	45.81 ± 1.27	43.67 ± 1.29
0.5 mg/kg	44.47 ± 1.84	42.83 ± 0.82	44.44 ± 0.90	46.11 ± 1.71	42.67 ± 2.46	46.38 ± 1.55
1.0 mg/kg	47.60 ± 3.84	41.50 ± 1.82	41.78 ± 2.23	41.42 ± 2.54	42.83 ± 0.89	41.25 ± 2.50
2.0 mg/kg	46.47 ± 1.92	45.47 ± 2.19	45.87 ± 2.28	48.00 ± 1.71*	47.31 ± 3.22	40.16 ± 3.10

A significant change in the PR interval was observed at 120 min after the administration of 2 mg/kg-DON. *: Significantly different from the control value ($P < 0.05$). DON: Deoxynivalenol. Control: the control group administered the vehicle injection. Each value is expressed as the mean ± S.D.

Table 2. QRS duration in the control and DON-groups

	Time (min) after administration with vehicle (control) or DON (0.5-2.0 mg/kg)					
	30	60	90	120	150	180
Control	16.00 ± 1.10	16.15 ± 0.98	16.41 ± 1.26	16.57 ± 1.32	18.00 ± 1.53	17.50 ± 1.22
0.5 mg/kg	16.60 ± 2.31	16.89 ± 2.04	17.61 ± 3.19	15.61 ± 1.26	18.39 ± 2.32	17.42 ± 2.13
1.0 mg/kg	17.44 ± 1.98	19.89 ± 3.76	15.61 ± 1.26	20.33 ± 2.21	15.89 ± 1.00	17.00 ± 1.34
2.0 mg/kg	23.33 ± 1.54	19.22 ± 1.32	22.83 ± 3.07	22.94 ± 2.37	20.93 ± 2.94	21.65 ± 3.28

No group showed a significant difference from the control. DON: Deoxynivalenol. Each value is expressed as the mean ± S.D. Control: the control group administered the vehicle injection.

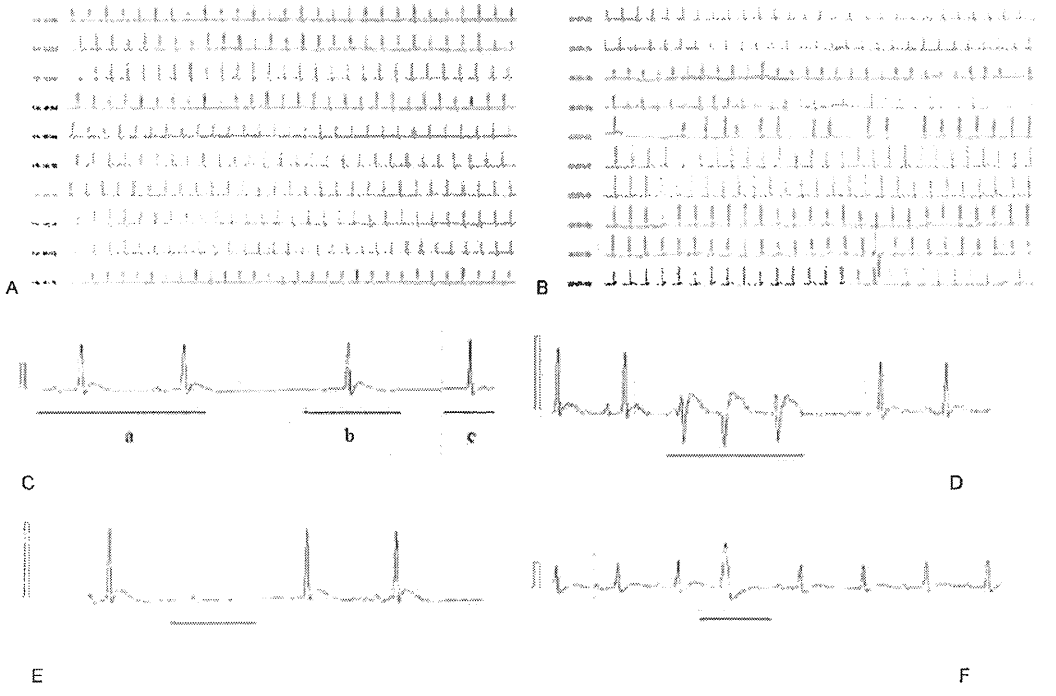

Fig. 4. Representative records of telemetric electrocardiogram (ECG). A: Normal ECG pattern before the injection of DON. B-F: Abnormal ECG pattern, including second-degree AV block after the DON-injection(B). C: Normal heart rhythm (a), Atrial bradycardia (b), Supraventricular extrasystole (c) D: Left ventricular extrasystole (short-run type). E: Second-degree AV block (underlined) F: Right ventricular extrasystole (underlined).

Table 3. QT interval in the control and DON-groups

	Time (min) after administration with vehicle (control) or DON (0.5-2.0 mg/kg)					
	30	60	90	120	150	180
Control	69.85 ± 2.11	69.62 ± 2.04	71.98 ± 2.88	73.71 ± 2.67	71.92 ± 2.57	71.57 ± 2.95
0.5 mg/kg	67.78 ± 1.93	70.33 ± 1.89	69.72 ± 2.98	69.44 ± 2.37	72.33 ± 3.54	65.58 ± 2.98
1.0 mg/kg	69.17 ± 2.03	71.78 ± 1.52	68.67 ± 3.62	70.28 ± 2.79	66.28 ± 3.97	67.29 ± 3.24
2.0 mg/kg	72.31 ± 4.61	80.67 ± 4.17*	78.28 ± 6.28	78.17 ± 4.06	71.94 ± 3.17	72.83 ± 3.59

A significant change in the QT interval was observed at 60 min after the administration of 2 mg/kg-DON. DON: Deoxynivalenol. Control: the control group administered the vehicle injection. *: Significantly different from the control value ($P < 0.05$). Each value is expressed as the mean ± S.D.

Table 4. Occurrence of arrhythmias in the control and DON-groups

Arrhythmia	Dose (mg/kg)			
	0	0.5	1.0	2.0
Second-degree A V block*	0/6 (0)	2/6 (0.5)	4/6 (1.2)	6/6 (2.3)
Supraventricular extrasystole	0/6 (0)	3/6 (0.8)	2/6 (0.6)	3/6 (2.0)
Ventricular extrasystole	0/6 (0)	4/6 (1.2)	5/6 (1.6)	5/6 (2.0)

A significant dose-dependent increase was observed in the incidence of second-degree AV block (*$P < 0.05$). No evidence of these arrhythmias was detected in the 0 mg/kg DON group (vehicle). The frequency of arrhythmias was calculated as the number of animals in which representative arrhythmias were identified (n = 6/group). The mean frequency of arrhythmias in each rat is shown in parentheses. DON: Deoxynivalenol.

DISCUSSION

The present study elucidated that the administration of DON induced significant cardiac toxicity: an increased heart rate, prolongation of the PR and QT intervals, and the occurrence of arrhythmia. Many of the components involved in heart rate regulation in normal rats are strongly influenced by the balance of autonomic nervous activity. Therefore, the increased heart rates observed in the 1.0 and 2.0 mg/kg-DON groups in the present study might have been due to either an increase in sympathetic nervous activity or a decrease in parasympathetic nervous activity. In a previous study, dogs subjected to intravenous injection with T-2 toxin (2.0 mg/kg), a member of the trichothecene group of mycotoxins, displayed an increased heart rate within 45 ± 15 min of the injection, while such changes in heart rate were inhibited by pretreatment with propranolol (Bubien and Woods, 1987). In the present study, HF power, an index of parasympathetic nervous activity, was decreased in the 2.0 mg/kg- and 1.0 mg/kg-DON groups after 90 min following the DON injections. However, the LF power and LF/HF ratio were significantly decreased during this period after the DON-injection in these groups. Such results from the power spectrum analysis of LF components were not consistent with the increased heart rate observed from 90 to 180 min after the DON-injection. Thus, the short-term increase in heart rate observed after the DON administration in this study might have been partly derived from the direct toxicity of DON against the heart in addition to the autonomic nervous effects on the heart induced by the attenuation of the HF component. This marked alteration of the power spectrum resulted in a potent decrease in total power in the 2.0 mg/kg- and 1.0 mg/kg-DON groups. This suggests that the entire outflow of the autonomic nervous system was inhibited in the rats administered high doses of DON at 90 min after the DON injection.

The minor extent of prolongation of PR interval and QT interval was observed at 60 min and 120 min after 2.0 mg/kg-DON injections. These changes may reflect weak toxic effects of DON on atrioventricular conduction system and cardiac muscles immediately after the administration with high dose of DON. It has recently been reported that high concentrations of DON (50, 100, or 200 mg/l) induced decreases in action potential parameters including a prolongation of the action potential duration (APD) and the maximum rate of depolarization (Vmax) in cultured cardiomyocytes isolated from neonatal rats (Peng and Yang, 2003). If such effects are exhibited in myocytes, it is likely that DON modifies cardiac ion chan-

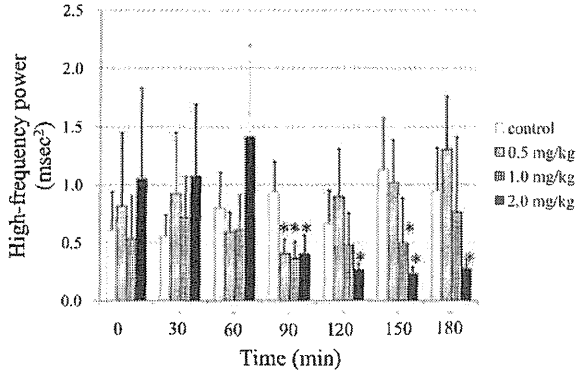

Fig. 5. Change in HF power after the DON-injection. The asterisks show significant differences at $P < 0.05$ (*) from the control value at each time point. Zero min is the time before the vehicle- or DON-injection. Data are expressed as the mean ± S.D.

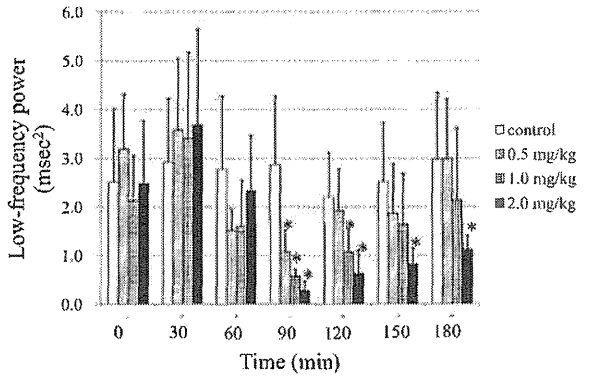

Fig. 6. Change in LF power after the DON-injection. The asterisks show significant differences at $P < 0.05$ (*) from the control value at each time point. Zero min is the time before the vehicle- or DON-injection. Data are expressed as the mean ± S.D.

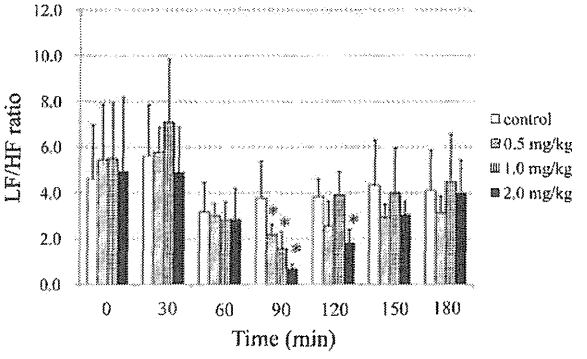

Fig. 7. Change in the LF/HF ratio after the DON-injection. The asterisks show significant differences at $P < 0.05$ (*) from the control value at each time point. Zero min is the time before the vehicle- or DON-injection. Data are expressed as the mean ± S.D.

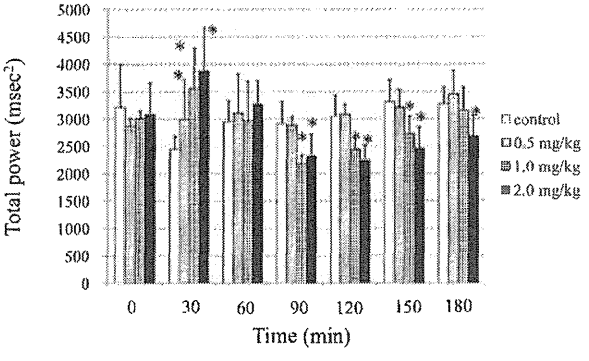

Fig. 8. Change in total power after the DON injection. The asterisks show significant differences at $P < 0.05$ (*) from the control value at each time point. Zero min is the time before the vehicle- or DON injection. Data are expressed as the Mean ± S.D.

nels involved in depolarization and/or repolarization, the intensity of which might depend on the concentration of DON administered.

In the present study, no obvious arrhythmias such as second-degree AV block, ventricular extrasystole, or supraventricular extrasystole were observed immediately after DON administration. Instead, they appeared after 3 hr, mostly at 10-20 hr after DON administration. This finding indicates that DON administration takes time to produce an arrhythmogenic condition in the rat heart. In a previous study in which the protein levels in the heart, liver, kidneys, and spleen were evaluated in mice that had been intraperitoneally injected with DON and sacrificed 5 hr later, it was found that DON with a dose of 20 or 80 mg/kg causes metabolic damage to myocytes by inhibiting protein synthesis in mice (Robbana-Barnat *et al.*, 1987). This study also demonstrated the histological changes represented by pericardial calcifications observed in most mice after 15 or 21 days of 20 ppm -DON ingestion.

In some studies, mitogen-activated protein kinases were suggested to play a role in the expression of DON-induced apoptosis (Baltriukiene, *et al.*, 2007; Pestka, 2008). Such intracellular metabolic toxicity might also

exist in myocytes, and it is feasible to suggest that the occurrence of arrhythmias, such as the premature ventricular contraction observed during the period from 10-20 hr after the DON administration, is, at least in part, associated with the cardiac toxicity caused by metabolic disorders in myocytes. Furthermore, it would be interesting to examine whether DON can induce apoptosis by producing oxidative stress in cells. A recent study examined DON-induced DNA damage in liver cells, by directly applying DON to a human hepatoma cell line (HepG2) and evaluating the DNA damage it caused by measuring the reactive oxygen species level in the HepG2 cells. As a result, it was suggested that the DNA damage induced by DON in the HepG2 cells was caused by oxidative stress (Zhang, 2009). Although it was not elucidated whether the DON administration in our study induced oxidative stress in myocytes, cardiac lesions caused by such mechanisms might have caused the arrhythmia observed in this study.

In conclusion, the results of the present study indicate that DON is acutely toxic to the heart at S.C. doses of 0.5 mg/kg and higher, disturbing the cardiac conduction and excitation system.

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RESEARCH ARTICLE

Reevaluation of arrhythmias and alterations of the autonomic nervous activity induced by T-2 toxin through telemetric measurements in unrestrained rats

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Abstract

This study was conducted to clarify and reevaluate the cardiac and autonomic nervous effects of T-2 toxin, which had been previously examined by several acute experiments, in unrestrained and conscious rats implanted with telemetric transmitters. Two groups of rats were given two subcutaneous injections of 0.1 and 0.5 mg/kg of T-2 toxin with an interval of 3 days. Two other groups of rat were pre-implanted with osmotic minipumps by which atropine (20 mg/kg/day) or propranolol (100 mg/kg/day) was continuously administered preceding subcutaneous injection of T-2 toxin (0.5 mg/kg). The present study demonstrated that T-2 toxin caused marked arrhythmias, such as second-degree atrioventricular (AV) block, sinus bradycardia, supraventricular extrasystole, and ventricular extrasystole, which were accompanied by a significant increase in heart rate and a significant decrease in total power and low- and high-frequency power of heart rate variability, during 3 days of observation after the toxin administration. However, the occurrence of arrhythmia with conduction disturbance such as second-degree atrioventricular blocks was markedly diminished by pretreatment with atropine, while the occurrence of ventricular extrasystole was augmented by atropine. The present study with the telemetric measurement elucidated and confirmed that T-2 toxin produced significant cardiac dysfunctions involving disturbance of the conduction pathway influenced by the autonomic nervous activity and also possible direct effects on cardiac myocytes.

Keywords: Cardiovascular, ECG, heart rate variability, mycotoxins

Introduction

The trichothecene group of mycotoxins has been known as a natural contaminant widely distributed in wheat, barley, tear grass, corn, cereal and their processed foods. This type of mycotoxin is produced by a variety of different fungi such as *Fusarium, Trichoderma, Myrothecium, Verticimonnosporium,* and *Stachynotrys* (Ballantyne et al. 2009). T-2 toxin is a representative of the trichothecene group (type A) of mycotoxins that may cause severe illness in animals and humans. Many common symptoms including fever, vomiting, leucopenia, hemorrhage, bone marrow depression, and hypertension or hypotension in humans and animals have been described as health effects of T-2 toxin (Sato et al. 1975; Weaver et al. 1978; Feuerstein et al. 1985; Ueno 1985, 1986; Borison and Goodheart 1989; Wannemacher et al. 1991).

Functional and morphological changes of the cardiovascular system have also been reported as effects of T-2 toxin. The cardiovascular changes, if they are potent, are noteworthy since such effects have not so frequently been described as health effects of other mycotoxins and arrhythmias such as ventricular tachycardia, if they occur, can be a lethal factor in both humans and animals. The previous studies on the cardiovascular system in experimental animals revealed a significant decrease in systemic blood pressure and

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systolic left ventricular pressure in anesthetized rats at 24 h following subcutaneous injection of 1 or 2 mg/kg of T-2 toxin (Magnuson et al. 1987) and also an increase in systemic blood pressure, arrhythmia lasting for 6–8 h and thereafter hypotension in rats administered T-2 toxin at doses ranging from 0.5 to 2 mg/kg (Feuerstein et al. 1985). Reflex effects of the autonomic nervous system on the cardiac conduction system and some inhibitory effects on action potential in the dog have been reported (Bubien and Woods 1986, 1987). In addition, it was shown that cultured myocytes ceased beating at 10–30 min after T-2 toxin application at concentrations of 250 μg/mL or higher (Yarom and Yagen 1986).

These findings suggested that T-2 toxin possesses a high possibility of cardiac toxicity in its nature. However, these previous studies were performed only under the acute stage of response observed mostly for a few or several hours in anesthetized animals or *in vitro* experiments, and there is little evidence about changes in cardiac and autonomic nervous activity for longer periods with consecutive recordings in conscious animals.

The aim of this study was to elucidate and reevaluate the toxic effects of T-2 toxin on cardiac and autonomic nervous functions through longer observation in unrestrained and conscious rats by a telemetric recording method and by use of pharmacological manipulations of the autonomic nervous system.

Materials and methods

Animals

The experiment was performed using 24 male Wistar rats purchased from Japan SLC, Inc. (Shizuoka, Japan) at 8 weeks of age and having the body weights of 230–250 g at 10 weeks of age, which is when the telemetric device was implanted. Each rat was maintained with *ad libitum* access to food and water in an individual cage within an isolation chamber maintained under controlled lighting (light-dark cycle, light = 12:00–24:00, dark = 24:00–12:00) and temperature conditions (24°C). All rats were fully adapted to these breeding environments during experiments.

Implantation of telemetry device for electrocardiogram (ECG) recording

An ECG telemetry recording system was employed in the present study in order to obtain information on cardiac function in unrestrained animals, including heart rate (HR), heart rhythm, and abnormal ECG waveforms by using implanted transmitters (TA10TA-F40, Data Sciences International, St. Paul, MN, USA). The implantation procedure including anesthesia and surgery was performed according to a previous study (Ngampongsa et al. 2011).

ECG signal acquisition and data analysis

One week after the surgery for implantation of the telemetry device, ECG signals were recorded from each rat in a cage that had been placed on a signal-receiving board (RA 1610, Data Sciences International, St. Paul, MN). The ECG data was sampled continuously at 1-m·s intervals, and all the data analyses, including fast Fourier transform (FFT) analysis and ECG wave component analyses, were performed using an ECG processor analyzing system (SBP2000U, Softron, Tokyo, Japan) equipped with a personal computer in series with an analog-digital converter; the ECG data were stored on an external hard disk.

The ECG wave components, i.e. PR interval, QRS duration, QT interval, and HR, were analysed for each 3 days before and after T-2 toxin or vehicle injection. Furthermore, the ECG waveform and heart rhythm (RR interval) were automatically or manually evaluated in order to detect episodes of arrhythmia before and after T-2 toxin administration.

In addition, the autonomic nervous function analysed by heart rate variability (HRV) was evaluated using software (SRV2W, Softron, Tokyo, Japan) on a personal computer through which the power spectrum was obtained by FFT analysis of the frequency component of the RR interval on ECG based on the Cooley-Tukey FFT algorithm (Cooley and Tukey 1965). In rats, there are two major spectral components, i.e. the low frequency (LF) (0.1–1.0 Hz) and high frequency (HF) (1.0–3.0 Hz) components, in the power spectrum analysis. A previous study using autonomic nervous blockades indicated that the LF component was influenced by both sympathetic and parasympathetic nervous activity and that the HF component was affected only by the parasympathetic nervous activity. Accordingly, the LF/HF ratio indicated the balance between the sympathetic and parasympathetic nervous activity (Kuwahara et al. 1994).

Injection protocol

All rats were randomly divided into four groups as described below. The T-2 toxin was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and dissolved in acetone/olive oil (5 μL/1 mL), and then the acetone was evaporated out under nitrogen gas for 30 min. The subcutaneous injection route was selected in the present study because the aim of the study was to observe cardiac and autonomic nervous responses to longer action of T-2 toxin, because subcutaneous injection of this toxin has been known to result in slow absorption through the skin, and because some tissue damage caused by such things as oxidative stress was shown to gradually increase to the maximum level at the third day after injection (Chaudhary and Rao 2010). Furthermore, the trichothecene group of mycotoxins usually produces strong gastrointestinal inflammation, anorexia, and in some animal species, vomit (Pang et al. 1987; Borison and Goodheart 1989), which may mask or interfere with the toxic nature of the mycotoxin in the extra-digestive organs. All injections were performed at 12:00, which is when the light period started, on each injection day. This injection time was selected to avoid large disturbances

in biorhythm resulting from handling stress due to injections in midway through the light or dark period. The doses for the injections in the present study were chosen by referring to the past reports on the cardiac effects of T-2 toxin (Feuerstein et al. 1985; Magnuson et al. 1987; Wang et al. 1998).

T-2 toxin at the doses of 0.1 ($n = 6$) and 0.5 ($n = 5$) mg/kg were subcutaneously injected twice at an interval of 3 days, to the rats at 11 weeks of age. Furthermore, 0.2 mL of olive oil as a vehicle control was given to the rats at 3 days before the administration of T-2 toxin in all experimental groups. In the present study, the cardiac effects of a higher dosage of T-2 toxin, e.g. 1 mg/kg, were not described because of early fatal termination during observation after administration.

Effects of the autonomic nervous blockades

Effects of atropine

Atropine sulfate (Tokyo Chemical Industry Co. Ltd.) dissolved into 0.9% NaCl-10 mM HCl (Carre et al. 1994) was used to observe the effects of parasympathetic blockade on the ECG changes caused by T-2 toxin. All 5 rats in this group received atropine infusion through an osmotic minipump (model 2ML1, Alzet Osmotic Pumps, DURECT Corporation, Cupertino, CA, U.S.A.) at a rate of 20 mg/kg/day for 7 consecutive days in which the dose of atropine was determined referencing to the previous reports (Carre et al. 1994; Brady et al. 1999). Before osmotic minipump implantation, 0.2 mL of olive oil was given as a control with vehicle. At the third day after implantation of the osmotic minipump, 0.5 mg/kg of T-2 toxin was administered to each rat in this group. No repeated injection of toxin was performed in this group.

Effects of propranolol

Propranolol hydrochloride (Tokyo Chemical Industry Co. Ltd.) dissolved into 0.9% NaCl-10 mM HCl (Carre et al. 1994) was used to observe the effects of sympathetic blockade on the ECG changes caused by T-2 toxin. All five rats in this group received propranolol infusion through an osmotic minipump (model 2ML1, Alzet) at a rate of 100 mg/kg/day for 7 consecutive days in which the dose of propranolol was determined referencing to the previous reports (Carre et al. 1994; Abe et al. 1999). At the third day after implantation, 0.5 mg/kg of T-2 toxin was administered to this group. No repeated injection of toxin was performed in this group. As described above in the experiment with atropine, 0.2 mL of olive oil was given before the T-2 toxin infusion.

Statistical analysis

All the results were statistically evaluated by two-way repeated-measures analysis of variance (two-way repeated-measures ANOVA) among all groups before and after vehicle or T-2 toxin injections. Comparisons between the T-2 toxin, "T-2 toxin + atropine," and "T-2 toxin + propranolol" groups were firstly performed with systemic random sampling and statistically evaluated by one-way non-repeated-measures analysis of variance (one-way ANOVA without replication) and two-way non-repeated-measures analysis of variance (two-way ANOVA without replication). In addition, Fisher's test was used to test significant differences between control and treatment groups for PR interval, QRS duration, QT interval, the occurrence of arrhythmia, and HR and HRV values. Differences with p values less than 0.05 were regarded as significant.

All experiments were conducted in accordance with the Animal Experimentation Guidelines of the University of Tokyo and approved by the institutional Animal Care and Use Committee of the Graduate School of Agricultural and Life Sciences at the University of Tokyo.

Results

Heart rate (HR)

Representative changes in HR that occurred during telemetric ECG recording are shown in Figure 1. A periodic alteration in HR that was dependent on the light-dark cycle was revealed (Figure 1A). Injection of T-2 toxin at concentrations of 0.1 and 0.5 mg/kg induced an increase in HR, which maintained a diurnal rhythm and the increased HR lasted for the three days observed after the first and second injections (Figure 1A and 1B). The changes in HR in response to the T-2 toxin administration are summarized in Figure 2. Significant differences ($p < 0.05$, two-way repeated-measures ANOVA) in HR were observed for order and doses of T-2 toxin injection. There was a significant increase in HR in the 0.5 mg/kg T-2 toxin groups ($p < 0.01$ or $p < 0.05$; Figure 2B) during the 3 days of observation, while the 0.1 mg/kg T-2 toxin group did not show any significant difference (Figure 2A). In the 0.5 mg/kg T-2 toxin group, HR significantly increased in the light period after both the first and second administrations ($p < 0.01$) compared with the control, while in the dark period, only the first administration showed an increase ($p < 0.05$). Also, the significant difference was found between the first and second administrations in both the light ($p < 0.05$) and dark periods ($p < 0.01$).

The alteration of HR in response to the autonomic nervous blockades, i.e. atropine and propranolol, is shown in Figure 2C. Atropine alone induced the increase in HR in the light period as compared with HR in the control (Figures 1C and 2C), while propranolol alone decreased HR in both the light and dark periods ($p < 0.05$) (Figures 1D and 2C). Comparing HR among the group with only T-2 toxin and two groups with autonomic nervous blockers, there was no significant change between T-2 toxin and T-2 toxin + atropine, while there was a significant decrease ($p < 0.01$) in the comparison between the T-2 toxin and T-2 toxin + propranolol groups in both the light and dark periods (Figure 2C).

PR interval

The changes in the PR interval observed after the administration of vehicle or T-2 toxin (0.1 and 0.5 mg/kg) are shown in Figure 3A. A significant decrease was revealed in the

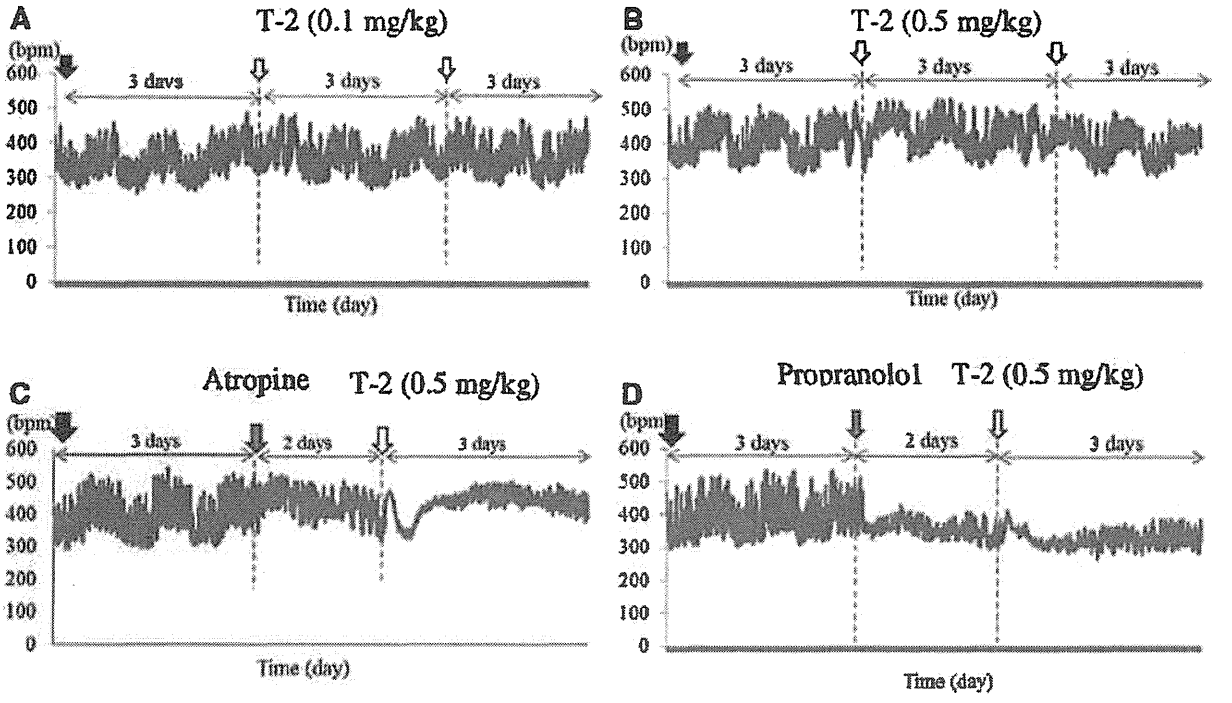

Figure 1. Representative changes in heart rate by recording telemetric ECG in rats. (A) 0.1 mg/kg T-2 toxin; (B) 0.5 mg/kg-T-2 toxin; (C) 0.5 mg/kg T-2 toxin + atropine (20 mg/kg/day); (D) 0.5 mg/kg T-2 toxin + propranolol (100 mg/kg/day). The arrows from the left to right in each panel indicate administration of vehicle (solid black arrow), intraperitoneal implantation of a minipump with blockades (shadow grey arrow), and administration of T-2 toxin (blank arrow).

0.5 mg/kg T-2 toxin group ($p < 0.05$), whereas the 0.1 mg/kg T-2 toxin group showed a significant increase in the second administration compared with the control ($p < 0.05$).

When the animals were pretreated with the blockers, there were no significant differences between the atropine and control groups, whereas propranolol alone significantly prolonged the PR interval ($p < 0.05$) (Figure 3D). Administration of the combination of T-2 toxin and atropine and the combination of T-2 toxin and propranolol significantly prolonged the PR interval ($p < 0.0.5$ and $p < 0.01$, respectively), as compared with T-2 toxin (0.5 mg/kg) alone (Figure 3D).

QRS duration

The changes in the QRS duration following vehicle or T-2 toxin (0.5 mg/kg) administration are shown in Figure 3B. The QRS duration was significantly prolonged in the T-2 toxin administration group at both the first and second injection ($p < 0.01$). The QRS duration was not altered by the presence of atropine (Figure 3E). The groups with both blockades exhibited significantly shorter QRS durations than the T-2 toxin group ($p < 0.05$) (Figure 3E).

QT interval

Changes in the QT interval observed after T-2 toxin administration are shown in Figure 3C. There were no differences in the QT interval at 0.1 mg/kg of T-2 toxin in both the first and second administrations compared with the control (Figure 3C). However, at 0.5 mg/kg T-2 toxin the QT interval was significantly decreased ($p < 0.05$) as compared with the control (Figure 3C). When compared between the first and second administrations, the QT interval was significantly prolonged at the 2nd injection ($p < 0.01$), while no significant change was recognized as compared with the control.

When atropine was pretreated, there was no significant change in any group. However, the QT interval was significantly prolonged by the pretreatment with propranolol alone ($p < 0.05$) and with the combination of T-2 toxin and propranolol ($p < 0.01$) when compared with the control (Figure 3F).

Occurrence of arrhythmia

T-2 toxin administration at all doses clearly induced arrhythmia, as represented by second-degree atrioventricular (AV) block (typically following first-degree AV block), ventricular extrasystole, ventricular tachycardia, supraventricular extrasystole, nodal escaped beat (parasystole), and sinus bradycardia (Figure 4). The episodes of these arrhythmias were mostly detectable from 8 h after toxin administration and lasted for a few days as shown in Figure 5. The same manner of occurrence of arrhythmia was recognized even at different doses. However, no obvious arrhythmias including ventricular extrasystole were observed in the control group.

The occurrence of arrhythmia during observation after T-2 toxin injection is summarized in Table 1. The frequency of episodes of second-degree AV block and

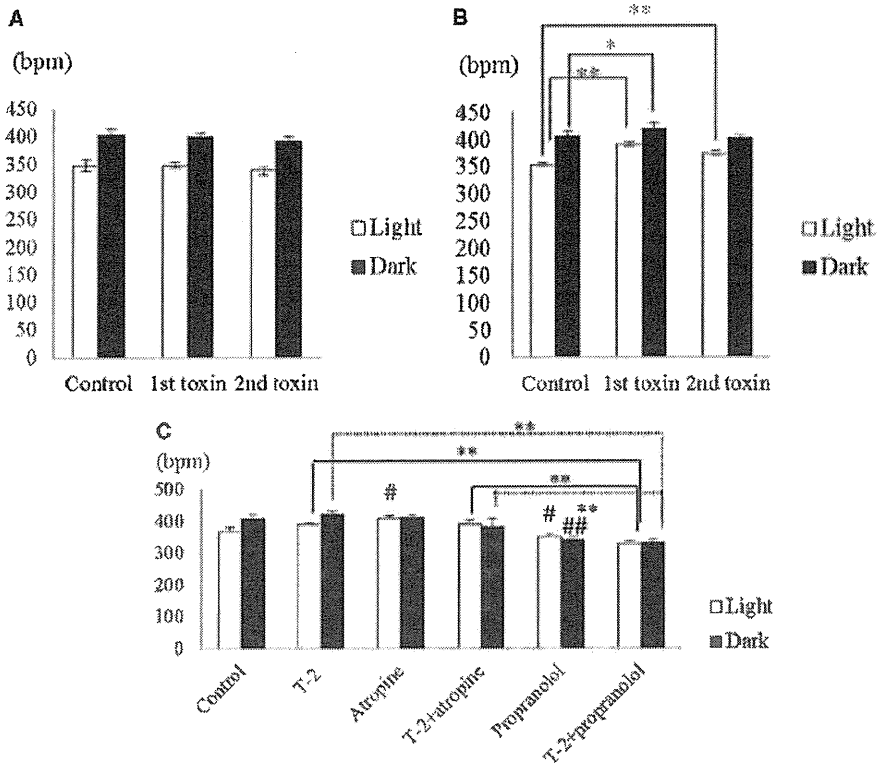

Figure 2. Changes in heart rate by injections of different doses of T-2 toxin and effects of autonomic nervous blockades. (A) 0.1 mg/kg of T-2 toxin ($n = 6$); (B) 0.5 mg/kg of T-2 toxin ($n = 5$); (C) A comparison among control (vehicle), T-2 toxin (0.5 mg/kg), blockades (atropine and propranolol), and T-2 toxin (0.5 mg/kg) with blockades ($n = 5$ in each condition). Asterisks: significant difference ($*p < 0.05$, $**p < 0.01$) between corresponding bars. Number signs: significant difference ($\#p < 0.05$, $\#\#p < 0.01$) from the control. Data are based on mean values obtained for each 2 or 3 days and expressed as means ± SEM.

sinus bradycardia increased significantly in a dose-dependent manner ($p < 0.01$). The overall occurrence of arrhythmias at 0.1 mg/kg was less than that at 0.5 mg/kg, being mainly due to no significant increase of second-degree AV block at 0.5 mg/kg. Each episode of arrhythmia usually lasted for several seconds. Significantly, supraventricular extrasystole appeared only in dose of 0.5 mg/kg T-2 toxin group ($p < 0.01$). A relatively large number of episodes of ventricular extrasystole (premature ventricular contraction) was observed and was mostly recognized at the lowest dose of T-2 toxin (0.1 mg/kg, s.c.) but not in a dose-dependent manner. In comparison of the first and second injections in the 0.5 mg/kg T-2 group, a significantly higher frequency of occurrence was found for the first administration of toxin ($p < 0.01$ and 0.05).

The effects of the two blockades are shown in Figure 6. Significantly, second-degree AV block and sinus bradycardia disappeared in both the "T-2 + atropine" and "T-2 + propranolol" groups ($p < 0.01$ and $p < 0.05$) (Figure 6A and 6D). Supraventricular extrasystole failed to show any significant changes when compared with other groups (Figure 6B). The combination of T-2 toxin and atropine significantly increased the occurrence of ventricular extrasystole when compared with the control group ($p < 0.05$) as shown in Figure 6C.

Low-frequency (LF) power

The changes in LF power observed after vehicle or T-2 toxin administration are shown in Figure 7A. A significant difference ($p < 0.05$, two-way repeated-measures ANOVA) was observed in only the light period in the 0.5 mg/kg T-2 toxin group, with marked decreases being recognized during the 3-day observation period as compared with the control group. Moreover, there was a significant difference between the 0.5 mg/kg T-2 toxin and 0.1 mg/kg T-2 toxin groups ($p < 0.01$) (Figure 7A). There was a marked decrease ($p < 0.01$) in LF power in the atropine group without T-2 toxin (Figure 8A). The existence of both blockades resulted in a significant decline ($p < 0.05$) in LF power as compared with the T-2 toxin group without blockades in both the light and dark periods (Figure 8A).

High-frequency (HF) power

Changes in HF power that occurred after vehicle or T-2 toxin administration are shown in Figure 7B. A significant difference ($p < 0.05$, two-way repeated-measures ANOVA) similar to that found in the LF power was found in the light period in the 0.5 mg/kg T-2 toxin group compared with the control group.

A marked decrease ($p < 0.01$) in HF power was recognized in the atropine group (Figure 8B) without T-2 toxin. In the T-2 toxin + atropine group, a significant decrease

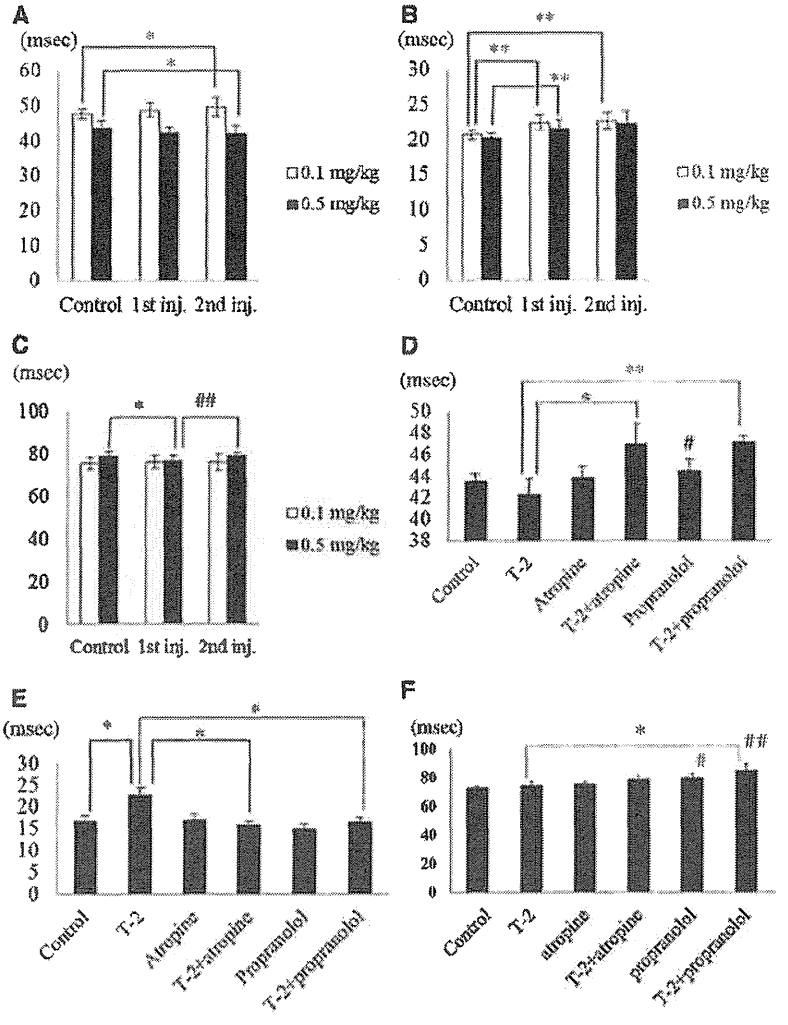

Figure 3. Changes in the interval components of ECG in response to T-2-injection. (A) PR interval; (B) QRS duration; (C) QT interval; (D) PR interval; (E) QRS duration; (F) QT interval. Asterisks in A to C: significant difference (*$p < 0.05$, **$p < 0.01$) from the control before T-2 toxin injection. Number signs in C: significant difference (##$p < 0.01$) between the first and second injection. Asterisks in D to F: significant difference (*$p < 0.05$, **$p < 0.01$) between the "T-2 toxin" and "T-2 toxin + atropine" or "T-2 toxin + propranolol" groups. Number signs in D to F: significant difference (#$p < 0.05$, ## $p < 0.01$) from the control. Data are based on mean values obtained for each 2 or 3 days and expressed as means ± SEM.

($p < 0.05$) in HF power in the light period was observed when compared with the T-2 toxin group without blockades (Figure 8B). There was no significant difference between the propranolol alone and T-2 + propranolol groups.

LF/HF ratio

The changes in the LF/HF ratio after vehicle or T-2 toxin administration are shown in Figure 7C. No significant differences were identified in this parameter.

With the blockades, LF/HF tended to decrease in every group except in the dark period in the atropine group, which showed a significant increase as compared with the control ($p < 0.05$) as shown in Figure 8C. Both the T-2 toxin + atropine and T-2 toxin + propranolol groups had significant decreases in the LF/HF ratio when compared with the T-2 toxin without blockades ($p < 0.05$ and 0.01, respectively) (Figure 8C).

Total power

The changes in total power that occurred after vehicle or T-2 toxin administration are shown in Figure 7D. A significant difference ($p < 0.01$, two-way repeated-measures ANOVA) was found in the 0.5 mg/kg T-2 toxin group but not in 0.1 mg/kg T-2 toxin group, with the total power being significantly decreased during the light period in 0.5 mg/kg T-2 toxin group ($p < 0.01$) compared with the control for 3-day observation period.

The total power significantly increased in the propranolol group in the dark period and further increased in the T-2 + propranolol group as shown in Figure 8D. In both the light and dark periods, T-2 toxin + propranolol produced a significant increase in total power when compared with T-2 toxin administration alone ($p < 0.01$).

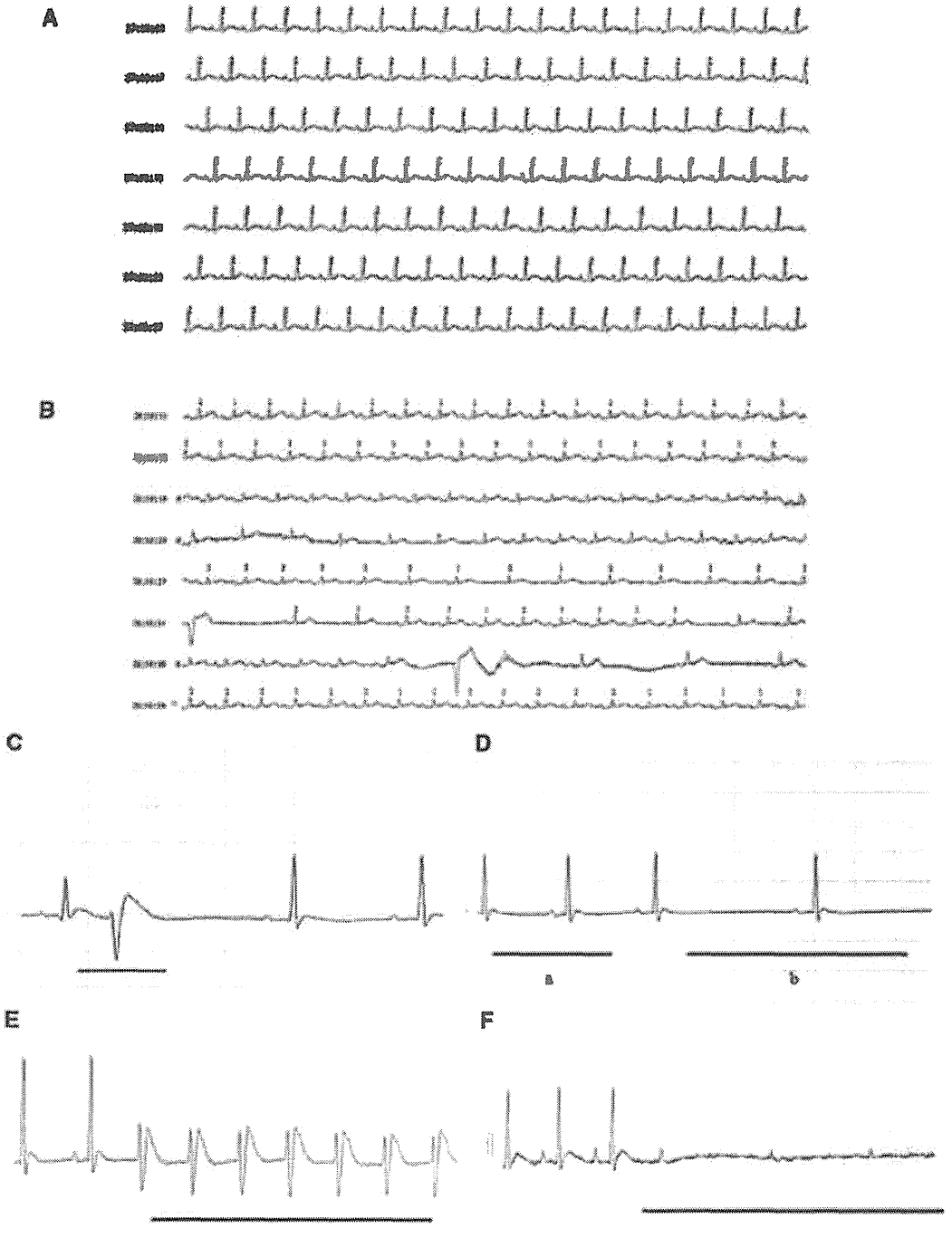

Figure 4. Representative records of telemetric electrocardiogram (ECG). (A) normal ECG pattern before the injection of T-2 toxin; B-F, abnormal ECG pattern; (B) second-degree AV block and ventricular extrasystole after the T-2 toxin injection; (C) ventricular extrasystole (underlined); (D) normal heart rhythm (a), sinus bradycardia (b); (E) ventricular tachycardia (underlined); (F) second-degree AV-block (underlined).

Discussion

It has been described by several studies with acute experiments using anesthetized laboratory animals or *in vitro* experiments that T-2 toxin has some cardiovascular toxicities; the majority of these studies were conducted in the 1980s (Schoental et al. 1979; Sherman et al. 1987; Borison and Goodheart 1989). The present study was performed to elucidate the properties of cardiac toxicity due to T-2 toxin in conscious and unrestrained rats using recent techniques involving telemetric measurement and power spectrum analysis of HRV, especially focusing on the

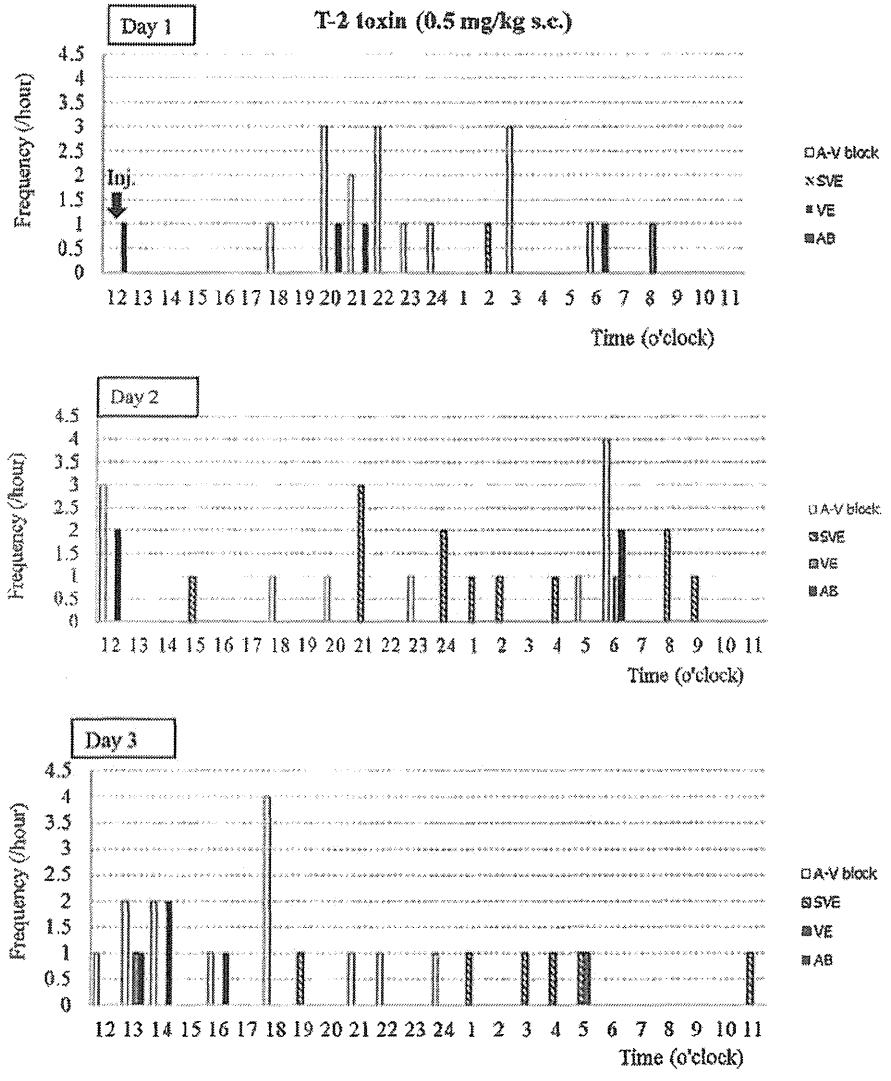

Figure 5. The occurrence of arrhythmia and time-course changes after administration of T-2 toxin. Data were obtained from all rats during 3 consecutive days after the 1st administration of T-2 toxin (0.5 mg/kg). AB, sinus bradycardia; A-V block, second-degree atrioventricular block; SVE, supraventricular extrasystole; VE, ventricular extrasystole and ventricular tachycardia.

Table 1. Occurrence of arrhythmias in the T-2 toxin groups.

	Dose (mg/kg)				
		0.1 ($n = 6$)		0.5 ($n = 5$)	
Type	Vehicle ($n = 11$)	1st inj.	2nd inj.	1st inj.	2nd inj.
Second-degree AV block	2.09 ± 0.81	2.83 ± 1.01	1.67 ± 1.28	7.8 ± 2.56**	4.2 ± 1.24#
Supraventricular extrasystole	0.55 ± 0.28	–	–	3.8 ± 2.58**	2.2 ± 1.71#
Ventricular extrasystole	0.09 ± 0.09	1.83 ± 0.48**	2.67 ± 1.43**	1.00 ± 0.45	–
Atrial bradycardia	0.27 ± 0.14	1.00 ± 0.37*	0.67 ± 0.49	2.4 ± 1.25**	1.2 ± 0.73##

The mean frequencies of arrhythmias in each rat were analysed for 3-day intervals and expressed as means ± SEM.
Significant differences (*$p < 0.05$, **$p < 0.01$) from the control before T-2 toxin injection.
Significant differences (#$p < 0.05$, ##$p < 0.01$) from values of the 1st injection.
–, No corresponding arrhythmia found.

appearance of arrhythmias, changes in ECG components, and alterations in the autonomic nervous activity.

The present study elucidated that the administration of T-2 toxin induced significant cardiac toxicity, that is, the frequent appearance of arrhythmia accompanied by an increased heart rate and the prolongation of QRS duration and QT interval during three days after toxin administration. The previous study by Bubien and Woods (1987) showed that anesthetized dogs subjected to intravenous injection with T-2 toxin (2.0 mg/kg) displayed a 17%

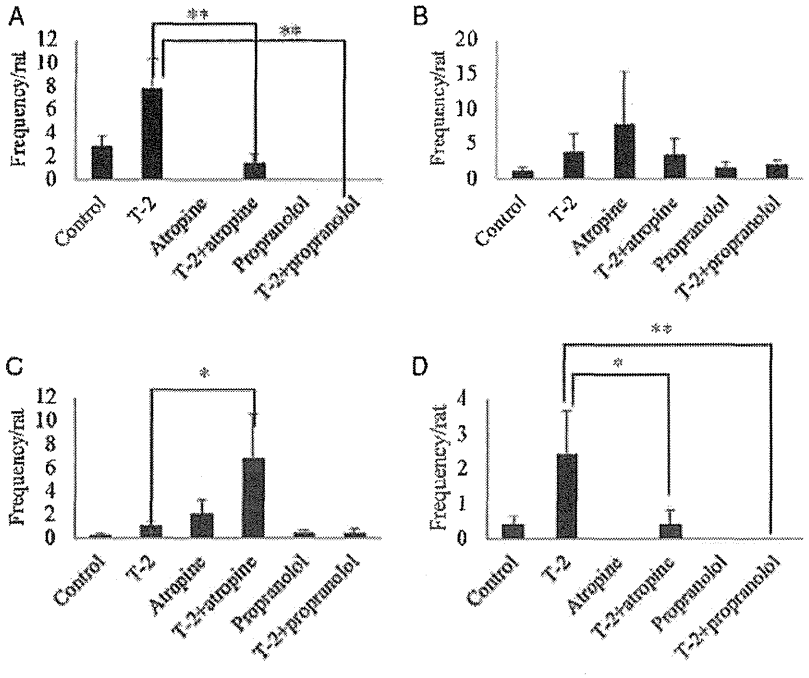

Figure 6. Change in arrhythmic occurrences response to 0.5 mg/kg T-2 toxin with two blockades (atropine 20 mg/kg/day or propranolol 100 mg/kg/day). (A) second-degree atrioventricular block; (B) supraventricular extrasystole; (C) ventricular extrasystole; (D) sinus bradycardia. Asterisks: significant difference (*$p < 0.05$, **$p < 0.01$) between the "T-2 toxin" group and "T-2 toxin + atropine" or "T-2 toxin + propranolol" group. Data are based on mean values obtained for each 2 or 3 days and expressed as means ± SEM.

increase in heart rate within 45 ± 15 min after injection that lasted for at least 4 h and were accompanied by decreased arterial blood pressure (92–83 mmHg). This study also revealed mild prolongation of cycle, i.e. slowing of heart rhythm, by recording action potentials from canine sinus node cells. On the other hand, Sirén and Feuerstein (1986) reported that in conscious rats measurement by a Doppler technique revealed a potent increase in vascular resistance and gradual decrease in blood flow during 6 h after intravenous injection of T-2 toxin at a dose of 1 mg/kg. These findings suggested that T-2 toxin could induce changes in blood pressure with hypertension or hypotension accompanied by a decrease or increase in heart rate after the administration of this toxin. Therefore, it should be considered that different cardiovascular alterations depending on the time course and animal species might be present in response to T-2 toxin. If hypertension due to increased vascular resistance is caused immediately after the administration of T-2 toxin, a decrease in heart rate could be elicited, and if hypotension, which may follow the initial hypertension, is caused, an increase in heart rate could be induced by the baroreflex mechanism. This suggests that the increased heart rate observed in the 0.5 mg/kg T-2 toxin group in the present study was due to sympathetic nervous activity since significant inhibition of the heart rate was produced by the preceding and continuous administration of propranolol. Although the extent of decrease in heart rate by propranolol was below the control level without T-2 toxin, especially in the dark period, it might be suggested from the result of the sympathetic blockade that the increased heart rate during the three days after administration did not seem to have originated from direct actions of T-2 toxin on the sinoatrial node.

The LF power, an index as both sympathetic and parasympathetic nervous activity, and the HF power, an index as the parasympathetic nervous activity, were significantly decreased by the administration of 0.5 mg/kg of T-2 toxin. The ratio of LF/HF power, an index of balance in the autonomic nervous activity, also significantly decreased at the same time. These changes in the HRV analysis did not indicate any predominant sympathetic nervous activity, although there was a trend of increase in LF/HF ratio at the dose of 0.1 mg/kg of T-2 toxin. Therefore, there was a discrepancy between the increase in heart rate and the results of HRV analysis at the dose of 0.5 mg/kg. This discrepancy might be partly due to the decrease in overall power at higher dose of T-2 toxin. In some conditions associated with sympathetic activation, the resulting tachycardia is usually accompanied by a marked reduction in total power, whereas the reverse occurs during vagal activation (Pomeranz et al. 1985). The marked alteration of the power spectrum suggests that the entire outflow of the autonomic nervous system was inhibited in rats administered a high dose of T-2 toxin. In the present study, the decrease in total power was remarkable in the light period. It is considered that the decreasing effect of the toxin on total power might be easier to observe in the light period than in dark period because the parasympathetic nervous tone, which

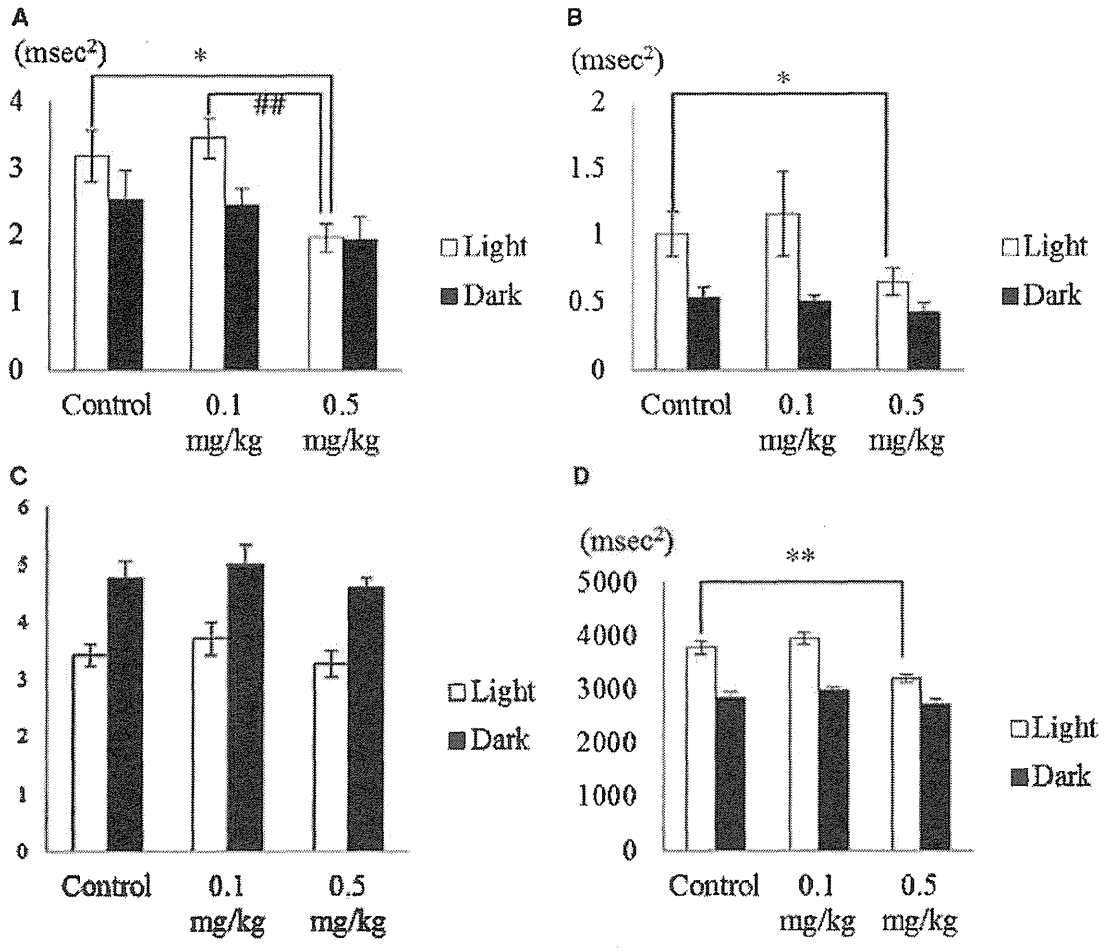

Figure 7. Changes in the value of power spectrum of heart rate variability in response to T-2 injection. (A) low-frequency (LF) power; (B) high-frequency (HF) power; (C) LF/HF ratio; (D) total power. Asterisks: significant differences (*$p < 0.05$, **$p < 0.01$) from the Number signs: significant difference (##$p < 0.01$) from the 0.1 mg/kg T-2 group. Data are based on mean values obtained for each 3 days and expressed as means ± SEM.

produces increases in HF and total powers, is basically high in the light period compared with the dark period. On the other hand, at the dose of 0.1 mg/kg of T-2 toxin, the LF/HF ratio was high in the dark period, suggesting that the autonomic nervous activity in rats with low concentrations of T-2 toxin is not largely influenced or somewhat tends to be sympathetic predominant.

A minor extent of prolongation and shortening of PR interval at 0.1 and 0.5 mg/kg of T-2 toxin was observed for the second injection of the toxin. As the PR interval indicates the function of the cardiac conduction system and the PR interval analysed in this study did not include the values recorded during second-degree AV block in which the R wave in ECG was absent, T-2 toxin may have some disturbing influences on cardiac conduction system. Moreover, significant prolongation of QRS duration was observed at 0.1 and 0.5 mg/kg of T-2 toxin. This change in QRS duration may be partly derived from direct toxic actions of T-2 toxin on the cardiac muscle since Bubien and Woods (1986) reported decreased resting potentials and action potential durations in the cardiac muscle cell in an experiment with recordings of canine heart tissues. However, in the present study, the significant shortening effect on QRS duration by pretreatment with both blockades indicated that the elongation of QRS duration by T-2 toxin alone was influenced by the autonomic nervous function. In general, it has been believed that the ventricular muscle is innervated by the sympathetic nerve but not by the parasympathetic nerve. A possibility that the shortening of QRS duration by atropine was associated with an increase in heart rate (shortening of the RR interval) was suggested. However, the QRS duration was also shortened by propranolol administration despite the heart rate being decreased by this drug. Mild intoxication due to continuous administration of the beta-blocker may be a possible explanation for this discrepancy since intoxication involves significant shortening of QRS duration as well as QT interval in humans (Love et al. 2002).

In the present study, with the exception of transient exhibition of sinus bradycardia, no obvious arrhythmias such as second-degree AV block, ventricular extrasystole, or supraventricular extrasystole were observed