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 (Bosh 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...
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 tri-chothecenes.

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,
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, 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.

**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
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 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 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 was also recognized at 120 min in the 2.0 mg/kg-DON group (P < 0.05).

**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.
Cardiac effects of DON

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

<table>
<thead>
<tr>
<th>Time (min) after administration with vehicle (control) or DON (0.5-2.0 mg/kg)</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>43.76 ± 1.42</td>
<td>43.33 ± 0.82</td>
<td>42.61 ± 1.19</td>
<td>42.15 ± 0.82</td>
<td>45.81 ± 1.27</td>
<td>43.67 ± 1.29</td>
</tr>
<tr>
<td>0.5 mg/kg</td>
<td>44.47 ± 1.84</td>
<td>42.83 ± 0.82</td>
<td>44.44 ± 0.90</td>
<td>46.11 ± 1.71</td>
<td>42.67 ± 2.46</td>
<td>46.38 ± 1.55</td>
</tr>
<tr>
<td>1.0 mg/kg</td>
<td>47.60 ± 3.84</td>
<td>41.50 ± 1.82</td>
<td>41.78 ± 2.23</td>
<td>41.42 ± 2.54</td>
<td>42.83 ± 0.89</td>
<td>41.25 ± 2.50</td>
</tr>
<tr>
<td>2.0 mg/kg</td>
<td>46.47 ± 1.92</td>
<td>45.47 ± 2.19</td>
<td>45.87 ± 2.28</td>
<td>48.05 ± 1.71*</td>
<td>47.31 ± 3.22</td>
<td>40.46 ± 3.10</td>
</tr>
</tbody>
</table>

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

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

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).
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 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

<table>
<thead>
<tr>
<th>Arrhythmia</th>
<th>Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Second-degree A V block*</td>
<td>0/6 (0)</td>
</tr>
<tr>
<td>Supraventricular extrasystole</td>
<td>0/6 (0)</td>
</tr>
<tr>
<td>Ventricular extrasystole</td>
<td>0/6 (0)</td>
</tr>
</tbody>
</table>

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.

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

<table>
<thead>
<tr>
<th>Time (min) after administration with vehicle (control) or DON (0.5-2.0 mg/kg)</th>
<th>0</th>
<th>0.5</th>
<th>1.0</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>69.85 ± 2.11</td>
<td>69.62 ± 2.04</td>
<td>71.98 ± 2.88</td>
<td>73.71 ± 2.67</td>
</tr>
<tr>
<td>0.5 mg/kg</td>
<td>67.78 ± 1.93</td>
<td>70.33 ± 1.89</td>
<td>69.72 ± 2.98</td>
<td>69.44 ± 2.37</td>
</tr>
<tr>
<td>1.0 mg/kg</td>
<td>69.17 ± 2.03</td>
<td>71.78 ± 1.52</td>
<td>68.67 ± 3.62</td>
<td>70.28 ± 2.79</td>
</tr>
<tr>
<td>2.0 mg/kg</td>
<td>72.31 ± 4.61</td>
<td>80.67 ± 4.17*</td>
<td>78.28 ± 6.28</td>
<td>78.17 ± 4.06</td>
</tr>
</tbody>
</table>

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.
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 (Baltriuikiene, 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.

ACKNOWLEDGMENTS

The authors would like to thank all the staff of the Laboratory of Comparative Pathophysiology and the Laboratory of Veterinary Public Health at the University of Tokyo for their kind support on the present study. This study was partly supported by a Grant-in-Aid from the Ministry of Health, Labor, and Welfare (H19-21, Food-General-009).

REFERENCES


