Prospective evaluation of corrected QT intervals and arrhythmias after exposure to epirubicin, cyclophosphamide, and 5-fluorouracil in women with breast cancer

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Background: Corrected QT (QTc) interval prolongation can induce fatal arrhythmias such as torsade de pointes.

Patients and methods: To assess the characteristics of QTc intervals and arrhythmias in women with early breast cancer who received FEC100 adjuvant chemotherapy, electrocardiograms (ECGs) were recorded before and after each chemotherapy. Associations between QTc interval prolongation and single nucleotide polymorphisms (SNPs) of potassium channel genes were also investigated.

Results: A total of 131 ECG records were obtained in 34 patients who received 153 cycles of FEC100. QTc intervals could be measured in 127 records. There was a significant trend toward QTc interval prolongation after each treatment, persisting through four cycles of chemotherapy (P < 0.001). Median QTc interval prolongations were 13, 11, 18, and 14 ms in the first through fourth cycles of chemotherapy, respectively. QTc intervals differed significantly between cycles 1 and 4 before treatment as well as after treatment (P < 0.05). A single supraventricular premature contraction was noted in 3 (2.3%) of the 131 cycles in 2 (5.9%) of the 34 patients. There was no significant association between QTc interval prolongation and SNPs of potassium channel genes.

Conclusion: This prospective study confirmed that FEC100 is associated with significant QTc interval prolongation in women with early breast cancer.

Key words: breast cancer, cardiovascular toxicity, chemotherapy, drug-induced QT prolongation

introduction

Cardiovascular toxicity has received increasing attention as an adverse event of pharmacotherapy, especially cancer chemotherapy. Besides acute coronary syndromes, thrombosis, hypertension, and arrhythmias, left ventricular dysfunction on echocardiography or multiple-gated acquisition scans is the most common type of cardiotoxicity. Left ventricular dysfunction can be caused by anthracycline antibiotics such as doxorubicin and epirubicin [1], HER2-targeting agents such as trastuzumab and lapatinib, and multi-targeted receptor tyrosine kinase inhibitors such as sunitinib and imatinib. Better management of adverse events associated with cancer chemotherapy is critical to preventing unnecessary toxicity, as well as to deriving maximal benefits from treatment.

Drug-induced QT interval prolongation has been recognized to be a clinically important type of cardiotoxicity, potentially leading to sudden death [2]. Since QTc interval prolongation can cause polymorphic ventricular tachycardia (i.e. torsade de pointes), resulting in fatal arrhythmias and sudden death, this adverse effect has received considerable attention over the past few decades. Unanticipated episodes of torsade de pointes and sudden death have led to the withdrawal of many drugs from the market, including terfenadine, mibebradil, sertindole, astemizole, grepafloxacin, and cisapride. Susceptibility to drug-induced QT interval prolongations has been recognized to be related to genetic mutations or single nucleotide polymorphisms (SNPs), some of which exist within potassium channel genes [3]. The relation of cancer chemotherapy to QTc interval prolongation and sudden death remains to be fully investigated [4–10]. One case report described three patients who died suddenly or had severe life-threatening arrhythmias during or immediately after treatment with doxorubicin [4]. In a retrospective study
of 52 survivors of childhood cancer who had received anthracycline derivatives, the 33 subjects who had received 300 mg/m² or higher doses of anthracyclines (doxorubicin-equivalent) had QTc interval prolongation more frequently than the 19 subjects who had received <300 mg/m² of anthracyclines, suggesting that persistent QTc interval prolongation is a measure of myocardial injury [5]. Another recent randomized study of early breast cancer reported two sudden deaths after adjuvant chemotherapy with epirubicin, cyclophosphamide, and 5-fluorouracil [6].

Fatal drug-induced toxicity must be carefully monitored, even when the frequency is very low. To gain insight into the relation between cancer chemotherapy and cardiotoxicity, we prospectively studied QTc intervals and arrhythmias by obtaining consecutive, standard 12-lead electrocardiograms (ECGs) in women with early breast cancer who received adjuvant chemotherapy with a combination of epirubicin, cyclophosphamide, and 5-fluorouracil (FEC100). We also analyzed SNPs of genes known to be associated with QTc interval prolongation to investigate the potential involvement of genetic factors.

patients and methods

patients

Women with early breast cancer who received FEC100 as adjuvant chemotherapy before or after surgery at Nagoya University Hospital were prospectively studied. All patients were treated in the outpatient chemotherapy center and gave written informed consent to participate in this study. The study protocol was approved by the Institutional Review Board of Nagoya University Hospital, and the study was conducted in accordance with the Declaration of Helsinki.

FEC100 regimen

The FEC100 regimen consisted of epirubicin 100 mg/m², cyclophosphamide 500 mg/m², and 5-fluorouracil 500 mg/m², given on day 1 of a 21-day cycle. Patients received four or six cycles, depending on their risk of relapse. Treatment was administered according to a standardized protocol. First, a 5-HT3 antagonist and dexamethasone 16 mg in 50 ml of normal saline were given as an i.v. infusion over the course of 15 min, followed by i.v. infusions of epirubicin 100 mg/m² in 50 ml of normal saline (15 min), cyclophosphamide 500 mg/m² in 250 ml of normal saline (60 min), and 5-fluorouracil 500 mg/m² in 50 ml of normal saline (15 min). At the end of the infusion, 50 ml of normal saline was administered as a flush over the course of 6 min.

electrocardiography and toxicity assessments

Standard 1-min, 12-lead ECGs were recorded three times after a 5-min bed rest, before (<10 min) and just after (<10 min) the first through fourth or sixth cycles of chemotherapy. The average of the three measurements was used for analysis. All ECGs were recorded by the same well-trained nurse throughout the study. QT intervals were measured by an automatic analyzing system included with the ECG unit. The accuracy of QT interval measurement and the presence or absence of arrhythmias was evaluated by two cardiologists who were blinded to patient information. QT intervals were adjusted with the use of both Bazett’s formula and Fridericia’s formula. In the former, the corrected QT interval is equal to the QT interval in seconds divided by the square root of the preceding RR interval (time interval between two QRS complexes) in seconds. In the latter, the corrected QT interval is equal to the QT interval divided by the cube root of the RR interval. The QT interval adjusted using Bazett’s formula was primarily analyzed in this study. QTc interval prolongation and other toxic effects were evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE), version 3.0. An expert panel of oncology nurses independently graded severity according to this scale in their daily practice to maintain consistency of ratings.

genotyping

Most studies of drug-induced QT interval prolongation have focused on genes of ion channels. Two large epidemiological studies reported that SNPs of potassium channel genes are associated with QTc interval prolongation [11, 12]. With the use of TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA), we investigated the following four SNPs within the potassium channel gene: KCNQ1-rs757092, KCNH2-rs1805123, KCNH2-rs3815459, and KCNH2-rs3807375. The KCNQ1 and KCNH2 genes encode the slow (I_{Ks}) and the rapid (I_{Kr}) components of the delayed rectifier current, respectively.

Genomic DNA was extracted from whole blood using a QiAamp Blood Mini kit (Qiagen, Valencia, CA), following the manufacturer’s protocol. The PCR mixture consisted of 10 ng of genomic DNA diluted in DNase- and RNase-free water, 10 μl TaqMan Universal PCR Master Mix (Applied Biosystems), and 1.0 μl of TaqMan SNP Genotyping Assays (assay ID: C_2990605_10, C_11631038_10, C_3219498_10, and C_2079799_20, respectively; Applied Biosystems), in a total volume of 20 μl on a 7300 Real-Time PCR System (Applied Biosystems). The PCR conditions were 95°C for 10 min, followed by 40 cycles of 92°C for 15 s, and 58°C for 1 min. The reaction plate was then read using a 7300 Real-Time PCR System instrument equipped with 7500 Fast System version 1.3.1 software (Applied Biosystems). For each polymorphism, a minimum of 10% of randomly selected DNA samples were sequenced to confirm the results and were subsequently used as controls.

statistical analysis

Differences in QTc intervals were tested with non-parametric Wilcoxon tests and Friedman tests for two and multiple samples, respectively. The Bonferroni correction was applied for multiple comparisons. Spearman’s rank correlation coefficient was used to analyze correlations between the numbers of the variant SNPs and the worst QTc interval prolongations. All statistical analyses were carried out with IBM SPSS Statistics 19 (IBM Japan Ltd, Tokyo, Japan).

results

From September 2007 through October 2008, a total of 34 Japanese women with early breast cancer received a total of 153 cycles of FEC100 regimen. Thirty-four patients gave informed consent for ECG recording in this study (Table 1). All of the patients had a performance status of zero. Echocardiographic assessments of left ventricular function were available for 26 (76%) of the 34 patients; the mean ejection fraction (± standard deviation) was 68.5 ± 5.3%. FEC100 was given to 26 patients (76%) after surgery and 8 (24%) patients before surgery. One patient with stage IV had metastases to para-aortic lymph nodes that were found after three cycles of neoadjuvant chemotherapy. No patient had hypokalemia, congestive heart failure, or bradycardia, major risk factors for QTc interval prolongation, at baseline examinations. In addition, no patient was receiving medications that potentially cause QTc interval prolongation, such as antiarrhythmics, tricyclic antidepressants, antipsychotic drugs, macrolide.
antibiotics, fluoroquinolones, and antifungal agents. A review of the medical history of the patients indicated that no patient had congenital hearing impairment or syncope or had a family history of congenital hearing impairment, syncope, or cardiac sudden death.

Chemotherapy was postponed in 17 (11%) of the 153 cycles (neutropenia, 14 cycles; febrile neutropenia, hepatotoxicity, and infection, 1 cycle each). Dose reduction was required during treatment in 7 (21%) of the 34 patients (febrile neutropenia, 6 patients; grade 4 anemia, 1 patient). No patient had symptoms or findings of left ventricular dysfunction during or immediately after chemotherapy. Chemotherapy was discontinued in two patients (anemia, one patient; disease progression, one patient). Neither QTc interval prolongation nor arrhythmias were associated with postponement of treatment, dose reduction, or discontinuation of chemotherapy.

**QTc interval prolongation and arrhythmias**

A total of 131 ECG records were obtained for 153 cycles of chemotherapy in 34 patients. QT intervals could be measured in 127 records but could not be evaluated in 4 records because of blurred T waves. All the 131 records were assessable for arrhythmias.

There was a significant trend toward QTc interval prolongation after each treatment, persisting through four cycles of chemotherapy ($P < 0.001$ for each cycle by the Wilcoxon test, Table 2; Figure 1). Median QTc interval prolongations were 13, 11, 18, and 14 ms in the first through fourth cycles of chemotherapy, respectively. Nine patients received five cycles of chemotherapy and 10 received six cycles. In these patients, QTc intervals were significantly prolonged after treatment in both the fifth ($P = 0.011$) and sixth cycles ($P = 0.028$). When QT intervals were adjusted using Fridericia’s formula, these results remained significant (data not shown).

According to the CTCAE grading, the worst grade of QTc interval prolongation after chemotherapy was grade 0 in 19 (56%) patients, grade 1 (QTc interval, $>0.45$ to $0.47$ s) in 10 patients (29%), and grade 2 ($>0.47$ to $0.50$ s or $>0.06$ s above baseline) in 5 patients (15%). No patient had grade 3 prolongation ($>0.50$ s). In 103 (81%) of the 127 records, the grading of QTc interval prolongation remained the same; 23 records (18%) showed a one-grade increase. One record (0.8%) obtained in the fourth cycle of treatment in a 42-year-old woman showed a two-grade increase (from grade 0 to grade 2).

Among the 16 patients in whom the ECG records were available for the first four cycles, QTc interval prolongation appeared to increase in parallel to the number of administered cycles. QTc interval prolongation differed significantly between cycles 1 and 4 before as well as after treatment ($P < 0.05$ for each by Wilcoxon test with Bonferroni correction) (Table 2; Figure 2). When the QT intervals were adjusted using Fridericia’s formula, similar trends toward QTc interval prolongation were observed, although the differences from cycles 1 to 4 were not significant ($P = 0.272$ and $P = 0.159$ by Friedman test before and after chemotherapy, respectively).

**Table 1.** Patients’ characteristics ($N = 34$)

<table>
<thead>
<tr>
<th>Age, years</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menopausal status</td>
<td>Pre</td>
<td>19 (56%)</td>
</tr>
<tr>
<td>Post</td>
<td>15 (44%)</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td>I–II</td>
<td>27 (79%)</td>
</tr>
<tr>
<td>III–IV</td>
<td>7 (21%)</td>
<td></td>
</tr>
<tr>
<td>N factor</td>
<td>Negative</td>
<td>22 (65%)</td>
</tr>
<tr>
<td>Positive</td>
<td>8 (24%)</td>
<td></td>
</tr>
<tr>
<td>Estrogen receptor</td>
<td>Positive</td>
<td>25 (74%)</td>
</tr>
<tr>
<td>Negative</td>
<td>9 (26%)</td>
<td></td>
</tr>
<tr>
<td>Progesterone receptor</td>
<td>Positive</td>
<td>5 (14%)</td>
</tr>
<tr>
<td>Negative</td>
<td>26 (76%)</td>
<td></td>
</tr>
<tr>
<td>HER2 status</td>
<td>Unknown</td>
<td>3 (9%)</td>
</tr>
</tbody>
</table>

**Table 2.** QTc intervals before and after chemotherapy

<table>
<thead>
<tr>
<th>Cycle</th>
<th>N</th>
<th>Before</th>
<th>After</th>
<th>Delta QTc</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>423, 411–428</td>
<td>436, 427–448</td>
<td>13</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>428, 418–433</td>
<td>439, 425–446</td>
<td>11</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>426, 418–439</td>
<td>444, 438–458</td>
<td>18</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>434, 421–448</td>
<td>448, 441–463</td>
<td>14</td>
<td>$&lt;0.001$</td>
</tr>
</tbody>
</table>

*QT intervals were adjusted using Bazett’s formula.

*Median, interquartile range (milliseconds).

*Delta QTc indicates median QTc interval prolongation after each treatment.
During the study, a single supraventricular premature contraction was noted in 3 (2.3%) of the 131 cycles in 2 (5.9%) of the 34 patients. No patient had ventricular premature contractions or other serious arrhythmias.

**QTc interval prolongation and SNPs**

Twenty-seven of the 34 patients (79%) gave informed consent for genetic analysis of potassium channel genes. There was no significant association between the occurrence of QTc interval prolongation and the SNPs of potassium channel genes: rs757092, rs1805123, and rs3815459. As regards rs3807375, a significant inverse association between the number of variant SNPs and the worst grade of QTc interval prolongation was observed, which was contrary to the previous report (data not shown) [12]. Genotyping results were as follows: KCNQ1-rs757092 was the reference A/A genotype in 11 (41%) patients, A/G genotype in 14 (52%), and G/G genotype in 2 (7%); KCNH2-rs1805123 was the reference A/A genotype in 27 (100%) patients; KCNH2-rs3815459 was the reference A/A genotype in 14 (52%) patients, A/G genotype in 10 (37%), and G/G genotype in 3 (11%); and KCNH2-rs3807375 was the reference A/A genotype in 17 (63%) patients, A/G genotype in 10 (37%), and G/G genotype in 0 (0%). When the genotyping results were transformed to a summation of the variant SNPs, there was no significant association between the number of variant SNPs and the worst prolongation of QTc interval ($r = 0.144, P = 0.474$ by Spearman’s rank correlation coefficient, $n = 27$).

**discussion**

The FEC100 regimen, a standard anthracycline-based regimen for chemotherapy against early breast cancer, consists of older drugs for which potential effects on QTc intervals were not assessed during clinical development. Although the major cardiotoxic effect of the FEC100 regimen as well as other anthracycline-based treatments is left ventricular dysfunction [1, 13], QTc interval prolongation has been recognized as another important toxic effect that may lead to sudden death [2, 7–10]. Our study showed that adjuvant chemotherapy with the FEC100 regimen was associated with significant QTc interval prolongation on ECG records in women with early breast cancer. Interestingly, two patterns of QTc interval prolongation were observed: (i) a transient prolongation of QTc interval after each dose of chemotherapy and (ii) a progressive increase in QTc interval prolongation in parallel to the number of treatment cycles administered. Neither of these patterns was accompanied by serious arrhythmic events. In a prospective study of 28 patients with non-Hodgkin’s lymphoma who received doxorubicin in a cumulative dose of

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**Figure 2.** QTc intervals from cycles 1 to 4. QTc interval prolongation increased in parallel to the numbers of treatment cycles and differed significantly between cycles 1 and 4 before ($P < 0.05$ by Wilcoxon test with Bonferroni correction) as well as after treatment ($P < 0.05$ by Wilcoxon test with Bonferroni correction). For each box plot, the box limits represent the 25th and 75th percentiles, the line within each box represents the median, and the whisker ends indicate the 10th and 90th percentiles.

**Figure 3.** The number of variant single nucleotide polymorphisms (SNPs) and QTc interval prolongation. There was no association between the number of variant SNPs and the worst prolongation of QT interval ($r = -0.144, P = 0.474$ by Spearman’s rank correlation coefficient, $n = 27$).
400–500 mg/m², QTc interval prolongation, QT dispersion, and late potentials developed independently of impairment of left ventricular function: QTc interval was significantly prolonged from 402.6 ± 4 ms at baseline to 416.6 ± 5 ms after treatment with doxorubicin in a cumulative dose of 500 mg/m², consistent with the cumulative QTc interval prolongation observed in our study [7]. In contrast to the transient QTc interval prolongation observed after each cycle of FEC100 chemotherapy, cumulative and irreversible prolongation of QTc intervals can subsequently have serious clinical consequences.

More than 10 genes have been found to be associated with congenital long QT syndromes and drug-induced QT interval prolongations. All of these genes are related to ion channels. Although four SNPs of potassium channel genes were studied, there was no convincing association between any SNP and the occurrence of QTc interval prolongation. Those who had apparent QTc interval prolongation might harbor other SNPs not assessed in this study. Because QTc intervals in our study were uniformly prolonged after chemotherapy and did not show any polymorphic variations, susceptibility to QTc interval prolongation after treatment with the FEC100 regimen is most likely not related to specific genetic variants.

The QTc interval prolongation in our study was most likely caused by the blockade of outward potassium currents in myocardial cells by chemotherapeutic agents, particularly epirubicin, during ventricular repolarization. The IKs current is known to be blocked by most drugs that cause torsade de pointes. However, the IKr current has been suggested to play a more important role in doxorubicin-induced QT prolongation [14]. The QTc interval prolongation induced by the FEC100 regimen might thus have different mechanisms and clinical consequences from those of other drug-induced QT prolongations.

Our study had several limitations, including a small number of patients, no serious arrhythmias, and lack of troponin tests. In addition, because follow-up ECGs and echocardiography were not mandatory, we were obliged to evaluate the long-term outcomes of prolonged QTc intervals retrospectively. Despite these limitations, we obtained follow-up ECG records from 13 (38%) of the 34 patients a median period of 133 days (range, 28–343 days) after the last treatment with FEC100. The median QTc interval was 434 s (interquartile range, 422–443 ms). Given the QTc intervals before cycle 4 (Table 2), our findings suggest that the QTc interval prolongations were sustained for a while after the completion of adjuvant chemotherapy. On the other hand, left ventricular function was assessed on follow-up echocardiography in 7 (21%) of the 34 patients. The mean ejection fraction (± standard deviation) was 67.9 ± 7.4%, suggesting no evidence of left ventricular dysfunction.

In conclusion, this prospective study demonstrated significant QTc interval prolongation on ECG records in patients with early breast cancer who received adjuvant chemotherapy with FEC100, a combination of epirubicin, cyclophosphamide, and 5-fluorouracil. The clinical consequences of such prolongation remain unclear. Given that most QTc interval prolongations remained within the same CTCAE grading and none were associated with any serious arrhythmias, future studies should include other measures of cardiotoxicity, such as left ventricular dysfunction and plasma troponin levels, to further delineate the clinical implications of QTc interval prolongation.

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disclosure

The authors declare no conflicts of interest.

references