POLYMORPHISM C3435T OF THE MDR-1 GENE PREDICTS RESPONSE TO PREOPERATIVE CHEMOTHERAPY IN LOCALLY ADVANCED BREAST CANCER WITH HER2/Neu EXPRESSION

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ABSTRACT

P-glycoprotein (mdr-1), encoded by the MDR-1 gene, confers multi-drug resistance against antineoplastic agents and important for the transmembrane transition of various other common therapeutic drugs. Recently, a polymorphism of MDR1 gene at cDNA position 3435 located in exon 26 has been shown to be correlated with clinical response to chemotherapy in cancer patients. The Anthracycline therapy, which is related to Her-2 expression in breast cancer patients, has better survival, although the mechanism of cancer cell sensitivity which expressed Her-2 to Anthracycline in its relation to P-glycoprotein expression is still unclear. The purpose of this study was to reveal those mechanisms mentioned above, which in the future could be used as the basis of Anthracycline application in breast cancer patients. An analytical observational research in nineteen patients diagnosed between January until December 2005 with locally advanced breast cancer treated by preoperative Anthracycline chemotherapy to evaluate its predictive outcome was performed. All samples were performed immunohistochemistry, PCR, and sequencing methodology of the MDR1 target gene. The results of this study showed that the polymorphism of MDR1 gene at cDNA position 3435 located in exon 26 has been shown to be correlated with clinical response to Anthracycline chemotherapy in breast cancer patients, without affected by positive or negative Her-2 expression. Patient with T/T genotype developed clinical response, while patient with C/T genotype did not develop clinical response. Thus, our study showed that the Breast cancer patients with positive Her-2 expression is not always responsive to Anthracycline application. It means that only patients with T/T genotype at position 3435 located in exon 26 of MDR1 gene that have clinical response, while patients who do not show clinical response having C/T genotype. MDR-1 polymorphism C3435T in exon 26 may co-determine resistance to chemotherapy and provide useful information to individualize therapy.

Keywords: Polymorphism C3435T, MDR-1, Her-2, Anthracycline, breast cancer

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INTRODUCTION

The MDR1 gene product P-glycoprotein (P-gp) is a member of the ATP-binding cassette transporter family. P-gp utilizes the energy derived from ATP hydrolysis to pump a wide range of compounds, including numerous clinically used drugs, out of cells. This activity has important pharmacokinetic and pharmacodynamic consequences. For example, P-gp is expressed within the apical membranes of intestinal, renal, and hepatic epithelial cells, where it affects the absorption and elimination of its substrates. P-gp is also located within the apical membranes of capillary endothelial cells of the brain, where it can limit the penetration of drugs to the CNS. In addition to the roles of P-gp in absorption, distribution, and elimination, the over-expression of P-gp is implicated in the development of the multi-drug resistance (MDR) phenotype of some tumor cells. Understanding the functional and clinical consequences of MDR1 variants is important. If this variability could be assigned to a mutation in the MDR1 gene, patients could be screened and appropriate dose adjustments could be made on the basis of their MDR1 genotype (Leonard et al. 2003; Longley & Johnston 2005). Recently, a number of papers have reported the discovery and initial characterization of MDR1 variants. To date, more than 20 mutations in the MDR1 gene have been identified. Until now, however, the functional and clinical consequences of only one common MDR1 variant, C3435T, have been investigated (Dresser 2001).
A polymorphism of MDR1 gene has been showed to be correlated with intestinal P-glycoprotein (P-gp) expression and activity in vivo (Hoffmeyer et al. 2000). This polymorphism consists of a C-to-T exchange at cDNA position 3435 located in exon 26 of the MDR1 gene. Although this base exchange does not affect the amino acid sequence of P-gp, the T allele appears to be associated with markedly lower MDR1 expression compared with the C allele (Nauck et al. 2000). Because of its silence on the protein level and its location in a non-regulatory region of the MDR1 gene, it is conceivable that this particular polymorphism is not causative for differences in P-gp expression. It is rather likely that this polymorphism is linked to other, as yet unidentified, changes in regions of the MDR1 gene that control expression, e.g., in the promoter/enhancer region or in regions that are relevant for mRNA processing. Nevertheless, the C3435T polymorphism appears to allow the differentiation of alleles with distinct MDR-1 expression and activity. Therefore, genotyping of the C3435T polymorphism may provide a basis for treating patients more effectively with agents that are substrates of the MDR-1 gene product, e.g., anticancer drugs such vincristine and doxorubicin (Nauck et al. 2000). The prevalence of breast cancer in Indonesia nowadays has not been known definitely. Breast cancer cases each year have a tendency to increase and most of them come with advanced stage.

The management of breast cancer patient now is improving progressively. For the last 2 decades, neoadjuvant or preoperative chemotherapy is developed to become one of the relatively new concepts in locally advanced breast cancer therapy. Many reports stated that the use of neoadjuvant chemotherapy in locally advanced stage breast cancer caused primary tumor regression around 60%-80%. Usually, Anthracycline group (epirubicin/doxorubicin), one of chemotherapy regiments, has good response, about 87%-91%.

Clinical tumor response to preoperative chemotherapy was graded as complete (cCR), partial (cPR), or no response (cNR). Patients who clinically responsive (operable) were operated and those who had no response were subjected to core biopsy. After that, immunohistochemistry, PCR, and sequencing of MDR1 target gene were performed to all samples.

MATERIALS AND METHODS

Between January until December 2005, nineteen patients diagnosed with locally advanced breast cancer were treated by preoperative anthracyline-based chemotherapy (3-4 cycles). Mean age of the patients that gave their informed consent to the study was 46.5 years (range 20-59) at the time of diagnosis. Tumor size was evaluated by physical examination, ultrasound and mammography at baseline before therapy, and after 3-4 course of chemotherapy or prior to the surgery. According to histopathological examination, all tumors were infiltrating ductal carcinomas. Evaluation of response to treatment was based on the recommended response evaluation criteria in solid tumors (RECIST). Clinical tumor response to preoperative chemotherapy was graded as complete (cCR) partial (cPR), or no response (cNR). Patients who clinically responsive were operated and those who had no response were subjected to core biopsy. After that, immunohistochemistry, PCR, and sequencing of MDR1 target gene were performed to all samples.

Nineteen specimens were obtained after chemotherapy. Immunohistochemical staining was performed using the avidin-biotin peroxidase kit (Vector Laboratories, Burlingame, CA). The sections were deparaffinized in xylene and rehydrated in serial ethanol. Endogenous peroxidase activity was blocked with methanol and 0.3% hydrogen peroxide. Slides were then incubated with 1.5% horse serum at 37° C for 20 minutes, and then incubated with monoclonal primary antibodies at 37° C for 30 minutes. Murine monoclonal antibody specific for p-glycoprotein, JSB-1 (Sanbio, Am Uden,
Polymorphism C3435T of The MDR-1 Gene Predicts Response to Preoperative Chemotherapy ... (Ami Ashariati)

Genomic DNA was isolated from patient’s tissue samples with a Qiagen DNA Blood Kit and a 106 bp fragment containing the C3435T polymorphism in exon 26 of the MDR-1 gene was amplified with the primer pair 5’-CCTATGGAGACAACAGCC-3’ / 5’-GAGAGACTTACATTAGGCAG-3’. The quality of PCR products was checked by agarose gel electrophoresis. Strands of the PCR product were separated by incubation of the beads with 50 µl 0.2 M NaOH for 1 min followed by two washes with 150 µl 10 mM Tris acetate.

RESULTS

Based on RECIST criteria, cCR was not observed but cPR in 14 (74%) resulting in an overall rate of clinical response to preoperative chemotherapy and 5 (26%) cNC was observed in the other patients. Based on immunohistochemical test, Her2 expression was observed in 11 (58%) of patients and 8 (42%) of patients showed negative Her2 expression. The genotypes of all 19 patients at position C3435 in exon 26 of MDR-1 gene were determined by PCR sequencing methodology. The C/T genotype occurred in 5 (26%) of the subjects, while the others were homozygous T/T (74%). Most of the patients (74%) with a T/T genotype responded clinically to preoperative chemotherapy, but the group possessing C/T genotype did not respond clinically to preoperative chemotherapy. Statistical analysis revealed non-significant correlation (p=1.000) between clinical response and the T/T genotype.

Table 1. The Characteristic of Breast Cancer patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median age (range)</th>
<th>Performance Status (ECOG) 100%</th>
<th>Tumor Size &amp; lymphnode status</th>
<th>Pathological characteristics</th>
<th>Pathological grade</th>
<th>Hormonal Receptors</th>
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<th>Total (%)</th>
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</thead>
<tbody>
<tr>
<td>Median age (range)</td>
<td>46.5 (20-59)</td>
<td>19 (100%)</td>
<td>T3 N1-2</td>
<td>Ductal invasive carcinoma</td>
<td>Grade III</td>
<td>ER+/PR+</td>
<td>ER+/PR-</td>
<td>11 (58)</td>
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<tr>
<td>Performance Status (ECOG) 100%</td>
<td></td>
<td></td>
<td>T4 N0-3</td>
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<td>ER-/PR+</td>
<td>ER-/PR-</td>
<td>8 (42)</td>
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<td>Tumor Size &amp; lymphnode status</td>
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<td>ER2 status</td>
<td>positive</td>
<td>11 (58%)</td>
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<td>Pathological characteristics</td>
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<td>8 (42%)</td>
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<td>Pathological grade</td>
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<td>Hormonal Receptors</td>
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<td>ER+/PR+</td>
<td>5 (26%)</td>
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<td>ER-/PR+</td>
<td>1 (5%)</td>
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<td>ER-/PR-</td>
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<td>Her2 status</td>
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Table 2. Distribution of Clinical Response and Her2 Expression

<table>
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<th>Expression</th>
<th>cResponse (+)</th>
<th>cResponse (-)</th>
<th>Total (%)</th>
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</thead>
<tbody>
<tr>
<td>Her2 (+)</td>
<td>8</td>
<td>3</td>
<td>11 (58)</td>
</tr>
<tr>
<td>Her2 (-)</td>
<td>6</td>
<td>2</td>
<td>8 (42)</td>
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<tr>
<td>Total (%)</td>
<td>14 (73.6)</td>
<td>5 (26.4)</td>
<td>19 (100)</td>
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(Fischer exact test p=1.000)

Figure 1. Electrophoregram MDR1 gene at position 3435 located in exon 26 of breast cancer from this study
DISCUSSION

The in vivo model of preoperative chemotherapy has proved to be useful to investigate novel therapeutic concepts and evaluate new predictive and prognostic factors. Bonadonna and Fisher et al. (as cited in Kafka et al. 2003), clearly demonstrated that response to preoperative chemotherapeutic treatment is an important indicator of both disease-free and overall survival. The identification of new predictor factors for response to preoperative chemotherapy may, therefore, help to define subgroups of patients that most likely benefit from this type of therapeutic approach.

Numerous drug resistance-associated genes have been described. However, their precise roles in the development of resistance to chemotherapy observed in the different types of human tumors still remain to be elucidated. This true even for the most extensively studied MDR-1 gene that was first discovered as a factor mediating multi-drug resistance in cancer chemotherapy. Its genes product P-gp was found to reduce the bioavailability of various compounds at the cellular level. Conferring multi-drug resistance to several types of tumors cells, MDR-1 expression and P-gp function is considered to contribute to cellular events that determine cancer patient’s resistance to chemotherapy (Kafka et al. 2003). Several other investigations (Duhem et al. 1996; Ejendal & Hrycyna 2002; Illmer 2002; Kafka et al. 2003) point to an implication of the MDR-1 genes in the manifestation of resistant phenotypes in locally advanced breast cancer treated by preoperative chemotherapy that co-determines the clinical outcome. Furthermore, MDR-1 expression induced by chemotherapy of locally advanced breast cancer was also found to be an indicator of a worse long-term prognosis. Kafka et al. (2003) assessed MDR-1 expression sequentially during preoperative chemotherapy and also demonstrated a significant correlation with response to treatment. If we take a good look, some theories saying mdr-1 expression is one of the factors that influence the chemotherapy resistance, are yet to be proven in this research. It is still unknown, whether this clinical response is caused by other factor, such as mdr-1 expression level that is encoded by MDR-1 gene, that might have an ability to determine which one is recognized matter or not and then being transported more effectively.

For the last 2 decades, neo-adjuvant or preoperative chemotherapy is developed to become one of the relatively new concepts in locally advanced breast cancer therapy. Many reports stated that the use of neo-adjuvant chemotherapy in locally advanced stage breast cancer caused primary tumor regression around 60%-80%. Usually, anthracycline group (epirubicin/doxorubicin) has good response, about 87%-91% (Campiglio & Somjenzi 2003; Childs et al. 2002; Clahsen et al. 1996). In one of the research result, patient with positive Her-2 expression are more responsive to anthracycline compared with negative Her-2 expression and statistically is significantly different. It was approved that Her-2 expression could be used as prediction biomarker in choosing regiment of medication of Anthracycline group or others (Clahsen et al. 1996). Although anthracycline gives better survival and there are relations between Her-2 expression with anthracycline sensitivity in breast cancer patient, the mechanism of cell cancer sensitivity which expressed Her-2 to anthracycline if it is related with MDR-1(P-gp) expression is still unclear.

One of the factors that influence chemotherapy-response is the variation at some MDR-1 genome area. Apparently MDR-1 gene located in exon 26 is found to have several variations. A polymorphism of MDR1 ...
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In Kafka et al. study (2003), rates of clinical and histopathological response to preoperative chemotherapy were comparable to the results of the other neoadjuvant trial. We determined the genotypes at position 3435 located in exon 26 of the MDR-1 gene in the patients. Remarkably, statistical analysis revealed a significant correlation between the T/T genotype and clinical response of the patients to preoperative chemotherapy. This correlation may be explained by genotype-dependent expression of P-gp that was demonstrated recently by Hoffmeyer et al. (2000). Although it is not obvious how expression is affected by the C3435T polymorphism that is not causing any variability in amino acid sequence (because C3435T is a silent mutation), MDR-1 expression in intestinal biopsies (n=21) was found to be 2-fold lower in individuals with a T/T genotype compared those possessing C/C genotype (Dresser 2001; Kafka et al. 2003). A similar correlation between final clinical outcome and MDR-1 exon 26 variants was recently reported for patients suffering from acute myeloid leukemia (Illmer 2002).

Our results showed that the difference in clinical response of breast cancer patients caused by variation of MDR-1 gene exon 26 on 3435 position, which can make difference in clinical response without affecting the positive or negative Her-2 expression. Patients with T/T genotype had clinical response, while patients who do not show clinical response having C/T genotype. Our results and other reports pointing to a correlation of single nucleotide polymorphism in the MDR-1 gene and expression or function of P-gp contribute to the information on genetic background that may be relevant to predict the individual outcome in therapeutic approaches with drugs that are affected by P-gp.

REFERENCES
