The effect analysis of CYP2D6 gene polymorphism in the toremifene and tamoxifen treatment in patient with breast cancer

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Abstract: The purpose of the present research work was to study the CYP2D6 gene polymorphism survival outcome after breast cancer patient received the toremifene and tamoxifen treatment. Seventy-eight patients who received radical mastectomy and toremifene and tamoxifen treatment after operation were divided into three groups: CYP2D6*1/*1 group (13 cases), CYP2D6*1/*10 group (28cases) and CYP2D6*10/*10 group (35 cases), according to the gene polymorphism of blood serum CYP2D6. The results of treatment of three groups were compared. After operation the content of blood serum CA125, CA153, VEGF, IGF-1 were all lower than before. The content of CYP2D6*10/*10 group was higher than those of CYP2D6*1/*1 group and CYP2D6*1/*10 group. The content of CYP2D6*1/*1 group had no difference with that of CYP2D6*1/*10 group. All patients were followed up for a median duration of 30.5 months. Progression-free survival (PFS) of CYP2D6*10/*10 was shortened. The recurrence rate increased and the survival rate reduced. There were no obvious differences between CYP2D6*1/*1group and CYP2D6*1/*10 group. In summary, CYP2D6 gene polymorphism relates with the effect of toremifene and tamoxifen treatment in patient with ER positive breast cancer and null allele homozygote CYP2D6*10/*10 can lead to a poor prognosis.

Keywords: Breast cancer, CYP2D6 gene polymorphism, toremifene, tamoxifen.

INTRODUCTION

Breast cancer is one of the most common malignancies with women in China, for which surgical resection combined with chemo-radiotherapy is the predominant procedure (Antonova *et al*., 2015; Avci *et al*., 2015; Babacan *et al*., 2015; Bal *et al*., 2015;Bozkurt *et al*., 2015; Bulut *et al*., 2015; Buyukhatipoglu *et al*., 2015; Yung *et al*., 2015). Toremifene (fig. 1) and tamoxifen (fig. 2 and fig. 3), belonging to selective estrogen receptor (ER) antagonist, are common medicines for endocrine therapy and suitable for ER positive patients. Through the competitive binding with the ER inside the tumor cells, these two medicines can block cell proliferation and differentiation. Although the endocrine treatment is quite effective on ER positive patient, its effect on patient with low drug sensitivity is not satisfactory due to the individual differences (Acharya *et al*., 2016; Li *et al*., 2016). Protein encoded by CYP2D6 gene (fig. 4) can catalyze toremifene and tamoxifen into Endoxifen with activity, of which the polymorphism may cause individual differences on effect of toremifene and tamoxifen. In recent years, it has been found that CYP2D6 allele *10 is one of the most common polymorphisms (SNPs) with a mutation rate of 57%. After mutation, its activity and metabolic conversion rate will reduce, influencing the toremifene and tamoxifen's conversion to the active form. The study is aimed at CYP2D6 Gene polymorphism in the toremifene and tamoxifen treatment in patients with breast cancer, providing testing basis for improving clinical chemotherapy effect.

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MATERIALS AND METHODS

Patients who were diagnosed as breast cancer in our hospital for the first time and received treatment from Jan 2013 to Jan 2016 were continuously selected, all with surgical resection indication and positive ER. Exclusion criterial: 1) patients who were pregnant or breastfeeding; 2) patients with primary tumors at other sites, metastatic breast cancer or underlying diseases such as severe cardiac, hepatic, renal, pulmonary or cerebral dysfunction. Shedding standard: (1) patient demanded to terminate the treatment; (2) patient was forced to terminate the treatment for failing to resist side effects of chemotherapy. Finally 78 patients with breast cancer were selected in the group with an average age of (58.4±7.9) years. TNM subsets were grouped into three stages: stage I (22 cases), stage II (31 cases) and stage III (25 cases).

Toremifene and tamoxifen were used for treatment after operation. Specific plans: Toremifene was taken 30mg/d and 1 time/d; tamoxifen was taken 10mg/d and 2 times/d. Follow-up of the tumor development was performed through telephone, text and out-patient review with follow-up rate of 100%. Progression-free survival (PFS), recurrence rate and survival rate were recorded. All the cases were followed up for 5~40 months with a median time of 30.5 months.

Five ml peripheral venous blood was collected on the second morning after admission. After ETDA anticoagulation, blood samples were centrifuged at 3000g for 15 min and the upper serum was taken and stored in the temperature of -20^{o}C. Plasma genomic DNA was

extracted with whole blood genome DNA isolation kit by TIANGEN Company and PCR amplification was performed with tetra-primer method. Amplification system included 1μl primer of each genomic DNA sample :5μl, 2×MasterMix12.5μl and 10μmol/L and 3.5μl deionized water; amplification condition were to perform the pre-denaturation at 95°C#for 10min and cyclic reaction at 94°C for 45s, 68°C#for 40s and 72°C#for 60s for 35 times. PCR reaction products were collected for agarose gel electrophoresis and gene segment was analyzed in the imaging system of agarose gel electrophoresis. Genotype CYP2D6*1/*1 had two products: 433bp and 180bp. Genotype CYP2D6*1/*10 had two products: 433bp and 290bp. Genotype CYP2D6*10/*10 had three products: 433bp、290bp and 180bp. Patients selected according to genotype differences were divided into three groups: CYP2D6*1/*1 group, CYP2D6*1/*10 group and CYP2D6*10/*10 group.

In three months before and after treatment, CA125 and CA153 content in serum were determined with automatic biochemistry analyzer, and VEGF and GF-1 content in serum were determined with ELISA kit. All procedures were strictly performed according to the instructions.

Data were recorded and analyzed by SPSS20.0 software. Measurement data were presented as mean ± standard deviation for statistical description. ANOVA analysis was performed in comparison among groups. Pairwise comparison was tested with LSD-t method. Comparison in the group was tested with paired T; count data were expressed in percentage and comparison among groups was subjected to (calibration) $\chi 2$ test; the difference with $P < 0.05$ was considered as statistically significant.

RESULTS

CYP2D6 Gene polymorphism

The number of cases in genotype CYP2D6*1/*1, genotype CYP2D6*1/*10 and genotype CYP2D6*10/*10 were respectively 13, 28 and 35 cases. Comparison of clinical information among three groups showed no statistically significant difference (P>0.05). See table1.

Serum markers of tumor

After treatment, the serum content CA125, CA153, VEGF and IGF-1 was lower than before. Content of CYP2D6 *10/*10 group was higher than those of CYP2D6 *1/*1group and CYP2D6*1/*10 group. There were no differences in comparison between CYP2D6*1/*1group and CYP2D6*1/*10 group. See table 2.

Follow-up survival outcome

Progression free survival in CYP2D6*10/*10 group was shortened with a higher recurrence rate and a lower survival rate; there were no differences in comparison between CYP2D6*1/*1group and CYP2D6*1/*10 group. See table 3.

DISCUSSION

Gene polymorphism in drug metabolism enzyme cytochrome P450 (CYP) can lead to differences in endocrine therapy effect on individual patient with breast

Cancer. As one of the most important members of CYP, CYP2D6 involves in metabolism of anti-depression drug, antiarrhythmic drug, analgesic and endocrine treatment drug (Ahmed *et al*., 2016; Carvajal *et al*., 2015; Caziuc *et al*., 2015; Cetean *et al*., 2015; Cetinkunar *et al*., 2015;

Chapoy-Villanueva *et al*., 2015;Chen *et al*., 2015; Cirak *et al*., 2015; Cvetanovic *et al*., 2015; Damyanov *et al*., 2015; Del *et al*., 2016; Hertz *et al*., 2016). Inside the body, toremifene and tamoxifen can be catalyzed and metabolized by CYP2D6 with the primary metabolite of 4-OH-TAM and secondary metabolite of 4-OH to methyl tamoxifen (Endoxifen) (Argalácsová *et al*., 2015). CYP2D6 Gene polymorphism influences CYP2D6 enzyme activity. When CYP2D6 catalytic activity reduces, the process that toremifene and tamoxifen catalyze into Endoxifen with CYP2D6 is blocked, reducing Endoxifen content in plasma (He *et al*., 2015). The affinity of Endoxifen for ER is 100 times stronger than tamoxifen and can effectively suppress independent cell proliferation of estrogen and its decrease in content can weaken endocrine disease specific drug's suppressing effect on independent cell proliferation of estrogen (Chin *et al*., 2015; Divani *et al*., 2015; Duzagac *et al*., 2015; Gao *et al*., 2015; Gatzidou *et al*., 2015; Gkialas *et al*., 2015; Guan *et al*., 2015; Gunaldi *et al*., 2015; Gu *et al*., 2015; Guo *et al*., 2015; Han *et al*., 2015).

At present, studies on CYP2D6 null allele have confirmed that polymorphism of locus *3-*8, *10-*16 and *18-*21 can result in CYP2D6 gene's not encoding functional protein, thus influencing enzyme activity (Dezentjé *et al*., 2015; Yazdi *et al*., 2015). The occurrence of homozygotes in the above mentioned null allele can cause enzyme losing its activity completely. The most common CYP2D6 null allele found in Asian women is CYP2D6*10 (Goldstein *et al*., 2015). In this study, heterozygotes occupied 35.90% (28/78), CYP2D6*10*10 homozygotes 44.87% (35/78) and wild-type CYP2D6*1*1 16.67% (13/78), indicating that CYP2D6 Gene polymorphism exists universally in patients with ER positive breast cancer. Occurrence rate of Null allele CYP2D6*10 was very high, and it can directly influence the process that CYP2D6 catalyzes toremifene and tamoxifen into Endoxifen.

The study has found that after treatment, the content of CA125, CA153, VEGF, IGF-1 in serum of patients in three groups was less than before. Content of CYP2D6*10/*10 group was higher than those of CYP2D6*1/* group and CYP2D6*1/*10 group. No

Table 1: Comparison of general clinical information of patients with different polymorphism of CYP2D6

Group	Number of cases	Age (years)	BMI (kg/m2)	TNM stages (I/II/III)	Max. diameter of tumor (mm)
CYP2D6*1/*1 group	13	58.9±8.2	22.1±3.5	4/6/3	12.3±3.5
CYP2D6*1/*10 group	28	57.6±7.2	22.5±3.3	8/10/10	13.2±3.6
CYP2D6*10/*10 group	35	58.2±7.7	21.8±3.1	10/15/11	12.5±3.4
F/χ2		0.235	0.352	0.785	0.158
P		0.862	0. 764	0.940	0.863

Table 2: Comparison of serum marker of tumors among three groups of patients

Group	CA125(U/ml)		CA153(U/ml)		VEGF(ng/ml)		IGF-1(ng/ml)	
	before treatment	after treatment	before treatment	after treatment	before treatment	after treatment	before treatment	after treatment
CYP2D6*1/*1 group	76.5±6.7	22.5±4.2	86.4±7.5	27.7±4.7	335.7±46.5	78.7±29.2	448.9±65.8	146.8±37.5
CYP2D6*1/*10group	77.2±7.3	25.1±3.9	86.3±7.9	27.1±5.1	346.2±47.2	80.3±21.0	465.7±72.3	155.1±39.2
CYP2D6*10/*10group	75.4±6.8	39.7±5.2	85.2±8.0	40.2±6.9	325.6±45.8	144.5±37.8	456.7±66.9	286.4±43.6

Table 3: Follow-up survival outcome of three groups of patients

Group	Number of cases	PFS (months)	Recurrence rate [n(%)]	Survival rate [n(%)]
CYP2D6*1/*1 Group	13	28.6±4.6	2(15.38)	11(84.62)
CYP2D6*1/*10 Group	28	27.03±4.2	3(10.71)	24(85.71)
CYP2D6*10/*10 Group	35	21.37±5.5	13(37.14)	20(57.14)

differences were seen in comparison between CYP2D6*1/*1 group and CYP2D6*1/*10 group (Abu 2017; Fang and Ruan 2017; Liu *et al*. 2017; Takahashi 2017). Detection of serum markers of tumor is one of the most common methods in clinical practices for early cancer screening and prognostic assessment. In the growth and proliferation of tumor cells, various molecules would be synthesized and secreted by tumor tissue and its host due to the existence of tumor, while the detection of the above mentioned markers would reflect proliferation conditions of tumor cells. CA125 and CA153 are two kinds of carbohydrate antigen which enters blood circulation in large quantities during the proliferation process of breast cancer; VEGF and IGF-1 can promote proliferation and growth, and the former can promote the angiogenesis while the latter can significantly promote the growth and division (Jafarpour-Sadegh *et al*., 2016; Li *et al*., 2016; Wang *et al*., 2016). Results showed stronger proliferation activity in breast cancer cells of patients in CYP2D6*10/*10 group, which indirectly reflected poor effect exerted by toremifene and tamoxifen in suppressing cell proliferation of CYP2D6*10/*10group. It was proved that null allele homozygotes influence the effect of toremifene and tamoxifen (Li *et al*., 2016).

In further follow up the survival outcome, it was proved that PFS of CYP2D6*10/*10 group was shortened with recurrence rate increased and survival rate reduced; there were no differences in comparison between CYP2D6 *1/*1 group and CYP2D6*1/*10 group. CYP2D6*10/*10 is the homozygote of null allele and enzymatic activity of CYP2D6 was lost completely, resulting in a decrease in treatment effectiveness of toremifene and tamoxifenin patient with ER positive breast cancer; CYP2D6*1/*10 is the heterozygote of null allele. Though enzymatic activity of CYP2D6 was lowered, it still can catalyze the production of Endoxifen. Toremifene and tamoxifen treatment in patient with ER positive breast cancer can still suppress cancer cell proliferation and improve prognosis of the patients.

CONCLUSION

In conclusion, CYP2D6 gene polymorphism relates with toremifene and tamoxifen treatment in patient with ER positive breast cancer and null allele homozygote CYP2D6*10/*10 can result in poor prognosis of the patients. Early detection of CYP2D6 gene polymorphism is expected to improve the long-term effect of chemotherapy.

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