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Association between polymorphism of dopamine D2 receptor genes and therapeutic effect of domperidone in functional dyspepsia

STOMACH

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Original Article

ABSTRACT

Background/Aims: Functional dyspepsia (FD) is a common gastrointestinal disease, for which domperidone is one of the most commonly used drugs. This study aimed to investigate the potential causes of the varying therapeutic effects of domperidone among FD patients.

Materials and Methods: The effects of domperidone therapy in patients with FD were evaluated using a clinical symptom score combined with real-time ultrasonography examination of antral motor index. Single nucleotide polymorphism (SNP) of the dopamine D_2 receptor genes 141C Ins/Del, A-241G, and TaqI, were analyzed by ligase detection reaction.

Results: The results of real-time ultrasonography correlated with clinical symptom scores. Single nucleotide polymorphism analysis of dopamine D_2 receptor TaqI gene showed that the genotype frequencies of "C/C", "C/T", and "T/T" in the effective group were 51.35%, 31.08%, and 17.57%, respectively; the allele frequencies were 66.89% and 33.11%. In the ineffective group, the genotype frequencies of "C/C", "C/T", and "T/T" were 17.81%, 52.05%, and 30.14%, respectively; and the allele frequencies were 43.84% and 56.16%. The difference was statistically different between the two groups ($p<0.01$).

Conclusion: Clinical symptom scores combined with real-time ultrasonography is effective in evaluating the therapeutic effect of domperidone in patients with functional dyspepsia. The therapeutic effect of domperidone in these patients was associated with polymorphism of dopamine D_2 receptor TaqI gene.

Keywords: Functional dyspepsia, dopamine D_2 receptor, genetic polymorphism

INTRODUCTION

Functional dyspepsia (FD) is a common gastrointestinal disease, which is described as a clinical syndrome with the postprandial satiety, upper abdominal pain or burning sensation, which cannot be explained by other pathologically based disorders (1). FD could be classified into two diagnostic categories according to ROME III criteria: postprandial distress syndrome and epigastric pain syndrome (2). The pathogenesis of FD is associated with fasting gastric motor disorder, postprandial abnormal food distribution in the stomach, abnormality of antrum-pylori tube-duodenal motor coordination, and delayed gastric emptying (3-5). Despite intensive studies over the past few years, the pathogenesis of FD remains unclear.

Gastric prokinetic agents, such as domperidone, specifically target dopamine D2 receptor and are one of the most used drugs for FD in the clinic (6). However, the efficacy of these agents for FD varies among patients (7). Other medications such as anti-acids, anti-anxiety agents, or drugs for gastric mucosa protection and *Helicobacter pylori* eradication were also reported to be ineffective for all FD patients (8). Thus, it may have significant clinical importance to elucidate potential reasons for variations in the therapeutic effect of domperidone among different FD patients.

Several methods have been developed to evaluate the therapeutic effect of different medications in FD patients. Most of these available methods are unpractical

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due to critical limitations such as cost, amount of time required, and low rate of compliance (9). Therefore, it is necessary to develop an effective, economic, and practical method to evaluate the therapeutic effect of different medications in FD patients.

In this study, we first evaluated the effects of domperidone therapy in FD patients using the clinical symptom score combined with real-time ultrasonography examination of antral motor index; then we analyzed single nucleotide polymorphism of dopamine D_2 receptor (DRD_2) gene 141C Ins/Del, A-241G and TaqI by ligase detection reaction to investigate the potential reason for the varied therapeutic effect of domperidone among different FD patients.

MATERIALS AND METHODS

Subjects

All subjects were outpatients between 18 and 65 years of age who visited Department of Gastroenterology, the First Affiliated Hospital, College of Medicine, Zhejiang University between 1st June, 2006 and 30th June, 2007 and fulfilled the diagnostic criteria for FD according to ROME III. The following patients were excluded: (1) those with a history of non-functional gastrointestinal disease within the last 4 weeks; (2) those with a history of hepatobiliary or pancreatic disease; and (3) those who are taking drugs, such as anticonvulsive drugs or any other kind of gastrokinetic compounds, that may influence the outcomes of the study. Informed consent was obtained from all of the subjects, and the study protocol was approved by the hospital ethics committee.

Clinical symptoms evaluation

Clinical symptoms, including early satiety, vague abdominal discomfort, nausea, occasional vomiting, epigastric pain or burning, belching, and postprandial fullness, were recorded at baseline and at 4 weeks after domperidone therapy. The grading of symptoms was performed as follows: grade 0 (none), no symptom; grade 1 (mild), slight discomfort and unawareness of symptoms, unless specifically focused; grade 2 (moderate), symptoms are obvious but daily work is not affected; grade 3 (severe), symptoms are severe, and daily work is affected. Considering that early satiety and vague abdominal discomfort are the two main complaints in FD, the scores for these symptoms were doubled. The total ameliorative rate of symptom (%) was calculated as follows:

ameliorative rate = (baseline score − post-treatment score)/ baseline score ×100%.

Domperidone therapy and outcome evaluation

All FD patients enrolled received standard domperidone therapy: 10 mg orally thrice a day for 4 weeks. The therapeutic outcomes were graded as follows: (1) full recovery, defined as disappearance of symptoms after therapy; (2) markedly effective, defined as symptom improvement by more than two grades; (3) effective, defined as symptom improvement by more than one grade; and (4) ineffective, defined as increased or unchanged symptom score after therapy. The subjects who were graded to have full recovery, markedly effective, and effective treatment outcomes were included in the effective group, whereas the remaining patients were included in the ineffective group.

Real-time ultrasonography examination

In order to evaluate gastric emptying, the following real-time ultrasonography parameters were recorded.

S_0: after abstaining from gastric prokinetic agents for 3 days and an overnight fast, the subjects were asked to sit on a chair and to lean slightly backwards, while an ultrasound probe was positioned vertically to permit simultaneous visualization of the antrum, the superior mesenteric artery, and the abdominal aorta. Antral area was estimated by tracing the mucosal side of the antrum with a built-in caliper; this area was designated as S_0.

The amplitude of contraction (ΔS): after instructing the patient to ingest 500 mL of 10% glucose within 5 min, an ultrasound probe was positioned vertically to permit simultaneous visualization of the antrum, the superior mesenteric artery, and the abdominal aorta. Immediately after ingestion, the antral area, in the relaxed and contracted state, was estimated six times by tracing the mucosal side of the antrum with a built-in caliper. The amplitude of contraction (ΔS) was calculated from the maximal reduction of the antral area during each contraction as follows.

ΔS = relaxed area minus contracted area.

Frequency: the total number of antral contractions were recorded for 4 min. The frequency (F) of antral contractions was defined as the average number of contractions per min.

Antral motor index: Antral motor index (MI) was expressed as the mean amplitude multiplied by the frequency of contractions, i.e., MI = ΔS×F. MI estimated by real-time ultrasonography is reportedly an accurate parameter for gastric emptying. Therefore, we applied this parameter in the present study to evaluate gastric emptying.

SNP analysis

Genomic DNA was isolated from peripheral blood mononuclear cells with the use of a DNA isolation kit (AXYGEN, AxyPrep-96 Blood Genomic DNA Kit). The DNA samples were run in 0.8% agarose gel by electrophoresis at 100V-150V and standardized to a final concentration of 50 ng/L. SNP sites of the DRD_2 gene 141C Ins/Del, A-241G, and TaqI were determined using ligase detection reaction.

The fragment that contains the SNP sites was amplified using PCR, using specific pairs of primers. PCR products were amplified in a Perkin-Elmer Gene Amp PCR Systems 9600 under the

Table 1. Comparison of the results of real-time ultrasonography in patients with functional dyspepsia after treatment with domperidone

	Effective group		Ineffective group	
Variables	**Pre-treatment**	**Post-treatment**	**Pre-treatment**	**Post-treatment**
Amplitude of contraction (cm^2)	416.2±22.1	515.4±21.6**	416.3±23.1	416.8±20.3##
Frequency of contraction (times/min)	2.03±0.23	2.74±0.27**	2.07±0.22	2.04±0.19##
Antral motor index	847.1±120.0	1410.9±154.8**	859.9±107.8	850.1±98.0##

**compared with pre-treatment in the effective group, p<0.01;
##compared with post-treatment in the effective group, p< 0.01

following conditions: initial denaturation at 95°C for 15 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 59°C for 60 s, extension at 72°C for 60 s, and a final extension at 72°C for 7 min. After the amplification reaction, the result of the PCR reaction was evaluated by adding 2 μL of the product to 3.0% agarose gel; this was electrophoresed in 0.5 × TBE buffer. The remaining samples were stored at -20°C for further use. The primers and probes used in this study are listed in Table 1, 2.

For each detection site, a pair of allele-discrimination oligonucleotide detection probes and a single oligonucleotide detection probe were designed. The discrimination oligonucleotide detection probe, containing 14 nucleotides that differ only at their 3′ terminal nucleotides, should complement the corresponding SNP target sequence, and should contain a biotin label at its 5′ end. The detection probe, containing 14 nucleotides with the sequence immediately adjacent to SNP site, was labeled with an FITC at its 3′ end and phosphorylated at its 5′ end for ligation reaction. When the target DNA forms a hybrid with the discriminating detection probes-detection probe, the ligation reactions start simultaneously during the LDR cycles in the presence of a thermo stable DNA ligase.

After denaturing the amplicon of PCR-LDR, the discrimination probe, the detector, and the ligase product were separated from the target template. The ligase product that had both biotin and FITC can form a complex with colored latex particles through a biotin-streptavidin interaction. The T-line has an anti-FITC antibody, so as the compound moves down the lateral-flow DNA strip, those with both biotin and FITC will be captured at the T-line to form a red line. In other words, perfect match reaction and single-base mismatch reaction can be distinguished by the color at the T-line; therefore, the genotype of the SNP could be easily identified. Gene types were determined by Gene mapper data analysis immediate diagram.

Statistical analysis

Continuous variables are presented as mean and standard deviation and were compared using Student's t-test; categorical variables were compared using the χ2 test. Statistical analyses were performed using the SPSS software package version 13.0 for Windows (SPSS Inc., Chicago, IL). A two-tailed p value of <0.05 was considered to be statistically significant.

RESULTS

Therapeutic effect evaluation

According to clinical symptom scores, a total of 74 patients with FD were classified in the effective group (21 males and 53 females; mean age, 41.50±9.80 years), and 73 FD patients were classified in the ineffective group (23 males and 50 females; mean age, 41.30±11.20 years).

Real-time ultrasonography

The results of real-time ultrasonography correlated with the clinical symptom scores (Table 1). In the effective group, domperidone therapy significantly increased the amplitude of contraction from 416.2±22.1 to 515.4±21.6 (p<0.01). Likewise, the frequency of contraction significantly increased from 2.03±0.23 to 2.74±0.27 (p<0.01). Most importantly, antral motor index significantly increased from 847.061±120.0 to 1410.9±154.8 (p<0.01) after domperidone therapy.

In the ineffective group, the amplitude of contraction, frequency of contraction, and MI were unaffected by domperidone therapy. The amplitude of contraction was 416.3±23.0 pre-treatment and 416.8±20.3 post-treatment (p>0.05); the frequency was 2.07±0.22 pre-treatment and 2.04±0.19 post-treatment (p>0.05); and the MI was 859.9±107.8 pre-treatment and 850.1±98.0 post-treatment (p>0.05).

SNP analysis

With regards the polymorphism of the DRD_2-141C Ins/Del gene, the genotype frequencies of "C/C", "C/-", and "-/-" in the effective group were 79.73%, 17.57%, and 2.70%, respectively and those in the ineffective group were 79.45%, 19.18%, and 1.37%, respectively (p>0.05). Also, the frequencies of the alleles were 88.51% and 11.49% in effective group and 89.04% and 10.96% in ineffective group (p>0.05).

With regards the polymorphism of DRD_2-A-241G gene, the genotype frequencies of "A/A", "A/G", and "G/G" in the effective group were 58.11%, 37.84%, and 4.05%, respectively; and those in the ineffective group were 61.64%, 35.62%, and 2.74%, respectively. The frequencies of the alleles were 77.03% and 22.97% in the effective group, and those in the ineffective group were 79.45% and 20.55%. The differences in genotype and frequencies of the alleles were not statistically significant between the two groups (p>0.05).

Table 2. Polymorphism of DRD2-TaqI in the two groups of functional dyspepsia patients after domperidone therapy

		Genotype (frequency)			Allele (frequency)	
Group	**Cases**	**C/C**	**C/T**	**T/T**	**C**	**T**
Effective group	74	38 (51.35%)	23 (31.08%)	13 (17.57%)	99 (66.89%)	49 (33.11%)
Ineffective group	73	13 (17.81%)	38 (52.05%)	22 (30.14%)	64 (43.84%)	82 (56.16%)
χ2		18.252			15.815	
p		<0.01			<0.01	

With regards the polymorphism of DRD_2-TaqI gene, the genotype frequencies of "C/C", "C/T", and "T/T" in the effective group were 51.35%, 31.08%, and 17.57%, respectively; and Those in the ineffective group were 17.81%, 52.05%, and 30.14%, respectively. The frequencies of the alleles were 66.89% and 33.11% in the effective group and 43.84% and 56.16% in the ineffective group. The differences were statistically significant between the two groups (Table 2).

DISCUSSION

In this study, we first evaluated the effects of domperidone therapy in FD patients using the clinical symptom score combined with real-time ultrasonography examination of the antral motor index and observed that the results of real-time ultrasonography correlated with the clinical symptom scores. Then, we analyzed polymorphisms of DRD_2, namely, 141C Ins/Del, A-241G, and TaqI and observed that DRD_2 TaqI polymorphism was associated with the therapeutic effect of domperidone in FD patients.

Functional dyspepsia is one of the most common gastrointestinal disorders encountered in clinical practice (1). The disease is estimated to affect about 15% of the general population in western countries and accounts for up to one-third of gastroenterology consultations (10). FD results in substantial health care cost, which directly includes doctor visits and expensive tests and medications and indirectly includes absence from work and low productivity at the workplace (5). The pathophysiology of FD is poorly understood. Recent studies showed that FD is a heterogeneous disorder wherein different pathophysiologic disturbances underlie different symptoms (11). Delayed gastric emptying and abnormalities of gastrointestinal motility have been observed in a considerable proportion of patients with FD (5). Medications aimed at improving delayed gastric emptying were observed to be effective for FD (12,13).

Several methods have been developed to evaluate gastric emptying (9). Scintigraphy is currently the only satisfactory method to quantitatively measure the rate of gastric emptying. However, the limitations of the lack of standardization of meal composition, patient positioning, timing of image acquisition, and lack of appropriate normal values with some meals inhibit widespread use of gastric emptying scintigraphy in the clinic (9). A new application of magnetic resonance imaging is also being developed for evaluation of gastric emptying. Magnetic resonance imaging has the advantage of providing comprehensive information, but it is quite expensive and requires further validation (9).

Functional ultrasonography is a relatively safe and inexpensive method to evaluate gastric function. Evaluation of gastric emptying by real-time ultrasonography was first described in the 1980s (14). Ultrasonography examination indirectly determines gastric emptying by real-time evaluation of changes in the antral area. Further studies showed that the values obtained with real-time ultrasonography did not differ grossly from those obtained with scintigraphy (15,16). In this study, our results showed that real-time ultrasonography significantly correlated with the clinical symptom scores of FD, and could be used as an objective method for the evaluation of FD.

Domperidone is commonly used for its prokinetic actions on the gastrointestinal tract. However, the efficacy of domperidone for FD varies among patients (7). Effectiveness of domperidone may be influenced by polymorphisms in genes that encode for drug-metabolizing enzymes, drug transporters, and domperidone targets (17). DRD_2 gene polymorphisms are considered the risk allele for antisocial phenotypes (18-20). However, the association between DRD_2 gene polymorphisms and FD remains unclear. In this study, FD patients with DRD_2-TaqI polymorphism who were effectively treated with domperidone showed a higher frequency of "C/C" genotype and a lower frequency of "T/T" genotype compared with those who were ineffectively treated by domperidone. DRD_2 TaqI polymorphism is considered to be associated with stress exposure (21), which may also play an important role in the development of FD. Our results indicated that the therapeutic effect of domperidone in FD patients was associated with polymorphism of DRD_2 TaqI. Further studies are needed to clarify the exact relationship between DRD_2 gene polymorphisms and the effect of domperidone therapy.

In conclusion, our results showed that the clinical symptom scores combined with real-time ultrasonography is effective in evaluating the therapeutic effect of domperidone in FD patients. Our results also showed that the therapeutic effect of domperidone in FD patients was associated with polymorphism of DRD_2 TaqI, but not with polymorphisms of DRD_2-141C Ins/Del and A-241G.

Conflicts of interest: The authors declare no conflict of interest.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of the First Affiliated Hospital, College of Medicine, Zhejiang University.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

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