β-2 Adrenergic receptor gene polymorphism and response to propranolol in cirrhosis

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AIM: To evaluate the association of β-2 adrenergic receptor (β2-AR) gene polymorphism with response of variceal pressure to propranolol in cirrhosis.

METHODS: Sixty-four non-related cirrhotic patients participated in this study and accepted variceal pressure measurement before and after propranolol administration. Polymorphism of the β2-AR gene was determined by directly sequencing of the polymerase chain reaction products from the DNA samples that were prepared from the patients.

RESULTS: The prevalence of Gly16-Glu/Gln27 and Arg16-Gln27 homozygotes, and compound heterozygotes was 29.7%, 10.9%, and 59.4%, respectively. Patients with cirrhosis with Gly16-Glu/Gln27 homozygotes had a greater decrease of variceal pressure after propranolol administration than those with Arg16-Gln27 homozygotes or with compound heterozygotes (22.4% ± 2.1%, 13.1% ± 2.7% and 12.5% ± 3.1%, respectively, P < 0.01).

CONCLUSION: The variceal pressure response to propranolol was associated with polymorphism of β2-AR gene. Patients with the Gly16-Glu/Gln27 homozygotes probably benefit from propranolol therapy.

Key words: Variceal bleeding; β2-adrenergic receptor; Propranolol; Variceal pressure; Homozygotes
Core tip: The study explored the influence of β-2 adrenergic receptor (β2-AR) polymorphism and the response of esophageal variceal pressure to chronic treatment with propranolol. The originality was that we associated the polymorphism to the measurement of variceal pressure and considered the response to propranolol administration. We found that the variceal pressure response to propranolol was associated with β2-AR gene polymorphisms, and that the patients with the Gly16-Glu/Gln27 homozygotes seem to benefit more from propranolol therapy.

INTRODUCTION

Variceal bleeding is a severe complication of patients with liver cirrhosis and portal hypertension. More than 40% of cirrhosis patients have esophageal varices at the time of diagnosis. Nearly 30% of those patients with large esophageal varices will bleed within 2 years[1]. Nonselective β-blockers are effective in preventing first variceal bleeding in patients with cirrhosis[2-4] because these drugs can reduce portal pressure[5,6]. Previous studies have reported that variceal bleeding can be effectively prevented by a decrease in hepatic venous pressure gradient (HVPG) < 12 mmHg after prophylactic propranolol therapy, or spontaneously[6,7]. Additionally, previous studies have demonstrated that patients with a decrease in HVPG from baseline of ≥ 20% have a low risk of first variceal bleeding and rebleeding[6,9], even if the final HVPG is > 12 mmHg[10-13].

Although the nonselective β-blockers decrease the portal pressure in cirrhosis patients, the response is not uniform. In a study involving 60 cirrhosis patients, 24 showed no reduction or even a slight increase in HVPG with propranolol[14]. Some patients who took the maximum tolerated dose of propranolol still had frequent bleeding, and did not display a significant decrease in the level of HVPG. Previous studies have found that β-2 adrenergic receptor (β2-AR) was polymorphic within the human population and that polymorphism of β2-AR gene plays a key role in modulating cardiovascular function. A more detailed study indicated that the two most common single nucleotide polymorphisms (SNPs) determine the hemodynamic response to propranolol occur at codons 46 and 79[11]. Several studies in healthy participants have shown that Gly16-Glu/Gln27 homozygotes express an upregulatory vasodilatory response to local infusions of receptor agonists, whereas Arg16-Gln27 homozygotes express a downregulatory vasodilatory response[15-17]. Patients with cirrhosis with Gly16-Glu/Gln27 homozygotes have a greater decrease in heart rate, cardiac index, and hepatic blood flow after propranolol administration than those with Arg16-Gln27 homozygotes. However, the HVPG responses to propranolol are similar in both groups[12]. Previous studies evaluated only an acute HVPG response to intravenous propranolol administration according to β2-AR gene SNPs, and did not take variceal pressure (VP) into account. VP is a major predictor of variceal bleeding risk; hence, it is an important marker of the response to pharmacological therapy in patients with portal hypertension[18-21].

Therefore, the aim of our study was to evaluate the association between VP response to propranolol and β2-AR gene polymorphism, and the prevalence of β2-AR gene polymorphisms in a small subgroup of patients with esophageal varices.

MATERIALS AND METHODS

Selection of patients

Between January 2010 and December 2012, a group of 64 cirrhotic patients (43 male and 21 female) were randomly selected to participate in the study. Their ages ranged from 18 to 70 years (median 50 years). All the patients were diagnosed with cirrhosis by liver biopsy and clinical, biochemical, endoscopic and ultrasonographic criteria. Esophageal varices were detected via upper gastrointestinal endoscopic examination. The causes of hepatic cirrhosis were hepatitis B virus (n = 51), alcohol (n = 7), cryptogeny (n = 5) and primary biliary cirrhosis (PBC, n = 1). Patients with the following criteria were excluded from the study: severe clotting defects, hepatic encephalopathy grade III and IV; Child-Pugh score > 12 points; multifocal hepatocellular carcinoma; contraindications to β-blocker therapy; pregnancy; or refusal to participate in the study. Patients with the VP < 15.2 mmHg were also excluded, along with patients who had undergone endoscopic interventions, including endoscopic variceal ligation and endoscopic injection sclerotherapy. The study was approved by the Ethics Committee of Anhui Medical University, and all patients gave written informed consent. A 2-mL venous blood sample was obtained from each patient and stored at -80 °C for further genotypic analysis.

Study design and VP measurement

Measurement of VP was performed after an overnight fast during upper gastrointestinal endoscopy. Somatostatin infusion was stopped 2 h before starting VP measurement. VP was assessed with a previously described noninvasive technique using an esophageal...
variceal manometer (EVM; Esophageal Varix Mano-
meter; Treier Endoscopy AG, Beromünster, Switzer-
land) and recorded by a workstation that was
developed by our team[22]. Before VP measurement, all
patients were sedated with 5 mg diazepam and 20 mg
n-butylscopolamine intravenously. In previous studies,
VP measured by this method had a good correlation
with that measured by needle puncture[23,24]. The
largest varix of the distal esophagus was chosen for
VP measurement. VP in each patient was measured
twice. VP was recorded as the mean of five
determinations that were taken during the procedure.

After VP measurement, the scales in the balloon
markers (5-mm intervals) were used to assess variceal
size. The maximal variceal size and esophageal
varical findings were reported as the Japanese Society
for portal hypertension[25]. After baseline measurement, propranolol was given orally
at an initial dose of 20 mg three times daily and was
increased by 20 mg every day over a period of 7 d
until the resting heart rate was reduced by 25%, or
was < 55 beats/min[41]. VP was assessed again at 7 d
of propranolol administration.

Two β2-AR gene functional SNPs were selected for
genotyping in this study: Arg16Gly and Gln27Glu.

Genotyping
Genomic DNA was extracted from the prefabricated
blood samples for genotype analysis. Two β2-AR SNPs,
Arg16Gly and Gln27Glu, were analyzed by allele-
specific polymerase chain reaction (AS-PCR). The
primers were designed as described previously[12].
For the Arg16Gly site, the upstream primer of Arg16 was 5'-CTTCTTCTGCTGGACCAATA-3', while
that of Gly16 was 5'-CTTCTGCTGGACCAATG-3', and
the downstream primer was 5'-CCAATTTAGG
AGATGTAAACTTC-3'. For analysis of the Gln27Glu
site, the following primers were designed, the
upstream primer of Gln27 was 5'-GGACCACGAC
GTCAGGCAG-3', and that of Gly27 was 5'-GGACCAC
GCAGGCAGG-3', and the downstream primer
of both was 5'-ACAATCCACACCATCAGAAT-3'.
The reaction was performed in a 50-μL mixture as follows:
DNA template 2 μL, each primer 1 μL, dNTP 1 μL, Pfu
dNA polymerase 1 μL, 10× Buffer 5 μL (containing Mg2+
20 mmol/L), deionized distilled water 39 μL. The
PCR was performed with an initial 94 ℃ for 5 min (pre-
degeneration), followed by 35 cycles (94 ℃ for 2 min,
degeneration; 55 ℃ for 1 min, annealing for Arg16Gly,
and 52 ℃ for 1 min for Gln27Glu; 72 ℃ for 1 min for polymerization),
and a final step at 72 ℃ for 10 min to
finish the reaction. The PCR products were separated
at 100 V for 50 min on a 1% agarose gel and were
visualized with ethidium bromide staining.

Calculation of sample size
Sample size was calculated to detect differences
between groups with different polymorphisms in
VP decrease from baseline of ≥ 10% after oral
propranolol, with a common variance of 40. With
an expected prevalence of 15% in the lower frequent
homozygotes (Arg16-Gln27) among the general
population[26,27], it was estimated that 47 patients
would be required in the study, to achieve 80% power
at the 5% level of significance.

Statistical analysis
Quantitative data were expressed as mean ± SD
and were compared using Student’s t test. One-way
ANOVA followed by pre-planned analysis was used to
compare the differences between the groups with
different polymorphisms. Comparisons of categorical
variables between different groups were performed
using Fisher’s exact test. Statistical analysis was
done using SPSS version 12.0 software. Statistical
significance was defined as P < 0.05.

RESULTS

Genotype analysis
We used the AS-PCR to test β2-AR SNPs in 64
individuals. The frequencies for three homozygotes
were Gly16/Glu27 = 28.1%, Gly16/Gln27 = 1.6%,
Arg16/Gln27 = 10.9%, and compound heterozygotes
= 59.4%. No significant differences were seen in
baseline characteristics between the groups of different
polymorphisms (Table 1).

Baseline VP and response to propranolol
All patients had severe portal hypertension as shown
by VP > 15.2 mmHg and the presence of esophageal
varices. No significant differences were seen in the
baseline VP among homozygous haplotypes (Table 1).

As expected, propranolol administration (80-160
mg/d, median: 120 mg/d) caused a significant
decrease in heart rate in each group. The median daily
dose of propranolol was 105 ± 34 mg in the Arg16-
Gly16-Glu homozygotes, and 108 ± 35 mg in the compound
homozygotes. There were no significant differences
among haplotypes (P > 0.05). We also found that
Gly16-Glu/Gln27 homozygotes had a greater reduction
in heart rate than Arg16-Gln27 homozygotes (-20.2%
± 1.4% vs -14.8% ± 2.2% respectively, P = 0.03)
(Table 2). Compound heterozygotes were found to
have intermediate response compared to those
homozygotes after oral propranolol treatment (-16.9%
± 2.9%).

As shown in Table 2, the reduction of VP was
significant after propranolol administration in each
group. The percentage VP reduction in the Gly16-
Glu/Gln27 homozygotes was significantly greater than
that in the Arg16-Gln27 homozygotes or compound
heterozygotes (22.4% ± 2.1%, 13.1% ± 2.7% and
12.5% ± 3.1%, respectively, P < 0.01).

**DISCUSSION**

Propranolol is a nonselective β-AR blocker and has been used to prevent variceal bleeding for many years. Propranolol prevents variceal bleeding and reduces HVPG via blocking β-AR to decrease cardiac output, heart rate and cardiac constriction, and via blocking β-AR to contract splanchic veins and reduce splanchic and portal blood flow.[13,14,28]. However, the effect of propranolol varies in different patients and the drug fails to reduce of HVPG level in some patients who have a high risk of bleeding and mortality.[29]. The discrepancy in the effect of propranolol in preventing variceal bleeding has attracted much research interest worldwide.[12,26,27,37]

Recently, in an attempt to explore the role of β-AR in the regulation of vascular tension and hemodynamic response to β-AR, it was found that β-AR gene polymorphisms played a key role in modulating cardiovascular function in humans.[31,32]. In particular, two common mutations of β-AR gene, +46 site G to A mutation and +79 site C to G mutation resulted in a change of amino acids of β-AR from Arg[16] to Gly[16] and Gln[27] to Glu[27], which played a little or no role in affecting the state of illness. However, it might affect the response of propranolol administration individually.[33,34]. Furthermore, it was found that homozygotes Gly[16]Gly or Glu[27]Glu genotype individual exhibit an enhanced vasodilatory response to isoproterenol infused through bronchial artery or arm vein locally.[16-17]. A similar result was obtained for therapy of asthma with β-adrenergic agents[35,36]. However, a study evaluating the role of β-AR gene SNPs in portal hypertension is still lacking. There are individual discrepancies in the preventive effect of propranolol on variceal bleeding that might be associated with β-AR SNPs. Patients with Gly[16]Gly or Gln[27]Glu homozygote genotype might benefit more from propranolol administration than those with Arg[16] or Gln[27] alleles.[12]. Nevertheless, that study only revealed an acute HVPG response to intravenous administration of propranolol, and the VP response to oral propranolol is still unknown. Previous studies have demonstrated that VP is a major predictor of variceal bleeding risk and the response to pharmacological therapy in patients with portal hypertension.[18-21]. For example, a VP level ≥ 15.2 mmHg represents a high risk of variceal bleeding in patients with cirrhosis[19]. Therefore, studies on the VP response to propranolol treatment have clinical significance.

The present study assessed the prevalence of β-AR gene polymorphism in a small subgroup of patients with cirrhosis. The prevalence of Gly[16]Glu/Gln[27] and Arg[16]Gln[27] homozygotes, and compound heterozygotes was 29.7%, 10.9%, and 59.4%, respectively. These data are similar to those in western studies and the US[12,26,27,37]. No significant

| Table 1 Demographic profile of the study population |
|-----------------------------------------------|
| **Arg**[16]**Gln**[27] (n = 7) | Compound heterozygotes (n = 38) | **Gly**[16]**Glu**/**Gln**[27] (n = 19) | *P* value |
| Sex (M/F) | 5/2 | 27/11 | 11/8 | 0.630 |
| Age (yr), mean ± SD | 51.60 ± 10.91 | 49.02 ± 22.52 | 51.95 ± 8.76 | 0.733 |
| Etiology | | | | |
| Hepatitis B | 5 | | | |
| Alcohol | 1 | | | |
| Cryptogenic | 1 | | | |
| Primary biliary cirrhosis | 0 | | | |
| Alcohol intake, Y/N | 0/7 | 6/32 | 3/16 | 0.756 |
| History of bleeding, Y/N | 2/5 | 8/30 | 5/14 | 0.858 |
| Ascites, Y/N | 0/7 | 11/27 | 5/14 | 0.332 |
| Albumin, g/L | 32.88 ± 5.85 | 32.36 ± 5.63 | 33.13 ± 4.72 | 0.668 |
| Total bilirubin (μmol/L) | 46.99 ± 35.92 | 40.76 ± 23.91 | 52.61 ± 69.65 | 0.072 |
| Prothrombin time (s) | 16.18 ± 2.23 | 15.84 ± 2.30 | 15.22 ± 2.56 | 0.713 |
| Serum sodium (mmol/L) | 138.50 ± 4.43 | 138.30 ± 4.74 | 139.12 ± 4.8 | 0.768 |
| Child-Pugh score | 6.86 ± 1.21 | 6.61 ± 1.52 | 6.74 ± 1.91 | 0.168 |
| VP (mmHg) | 21.35 ± 3.02 | 22.08 ± 3.26 | 21.69 ± 2.78 | 0.367 |

*P < 0.05, *P < 0.01 vs baseline. HR: Heart rate.

| Table 2 Heart rate and VP changes after propranolol according to β-AR gene polymorphisms |
|-----------------------------------------------|
| Variables | **Gly**[16]**Glu**/**Gln**[27] (n = 19) | Compound heterozygotes (n = 38) | **Arg**[16]**Gln**[27] (n = 7) |
| | Baseline | 7 d | Baseline | 7 d | Baseline | 7 d |
| HR (beats/min) | 76.3 ± 2.7 | 60.5 ± 1.8 | 75.6 ± 4.9 | 62.5 ± 3.6 | 74.1 ± 4.7 | 62.9 ± 3.0 |
| VP (mmHg) | 21.35 ± 3.02 | 16.52 ± 1.87 | 22.08 ± 3.26 | 19.43 ± 3.12 | 21.69 ± 2.78 | 18.79 ± 3.15 |

[22]
differences in the basal heart rate and VP regarding the different β2-AR haplotypes were found before propranolol administration. An important result from our study was that patients with cirrhosis and portal hypertension showed different responses to propranolol, as calculated by the reduction in VP. After administration of propranolol, patients with the Gly16-Glu/Gln27 homozygotes showed a greater reduction in VP, whereas patients with Arg16-Gln27 homozygotes exhibited a lesser reduction. The individuals who were compound heterozygotes had an intermediate response between Gly16-Glu/Gln27 and Arg16-Gln27 homozygotes.

The limitation of our study was that prevalence of β2-AR gene polymorphisms was investigated in a small subgroup of patients with cirrhosis, so, the assessment was not accurate. A prospective follow-up study of cirrhosis patients is underway to investigate the prevalence of β2-AR gene polymorphisms and analyze the impact of the polymorphisms on the hemodynamic effect of propranolol in esophageal varices.

In summary, we discovered that the individual differentiation of the effect of propranolol is associated with β2-AR 46 SNP. The replacement of amino acid 16 in the receptor from Arg to Gly results in an enhanced response to propranolol. Patients with an allele gene Gly benefit more from propranolol therapy than those with an Arg in long-term treatment.

ACKNOWLEDGMENTS

We would like to thank Professor Qiyi Tang, from the Department of Microbiology/AIDS program at Ponce School of Medicine, for language assistance.

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