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Format for ANSWERING REVIEWERS

Jan 25, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 14848-review.doc).

Title: The association between the effects of propranolol reduces variceal pressure and beta-2 adrenergic receptor gene polymorphism in Chinese cirrhotic patients

Author: De-Run Kong, Jin-Guang Wang, Chen Chen, Fang-Fang Yu, Qiong Wu, Jian-Ming Xu

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 14848

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Reviewer code: 00013649

Q The study explores the influence of beta-2 receptor polymorphism and the response of esophageal variceal pressure to chronic treatment with

propranolol. The originality is that authors associate the polymorphism to the measurement of variceal pressure and consider the response to chronic administration of propranolol. The topic is of interest, however some criticism may be moved as following detailed:

MAJOR POINTS

1-authors should describe the dose of propranolol in each polymorphism group (genotype homozygous for Arg16-Gln27, homozygous for Gly16-Glu/Gln27 or compound heterozygous) since the dose can influence the final response of the hemodynamic parameters considered in the analysis. Moreover the influence of the polymorphism on variceal pressure and the other hemodymnamic parameters should be adjusted for those clinical variables on uniformly distributed between groups of polymorphism**.**

2- Patients homozygous for Gly16-Glu/Gln27 haplotype should be those with the highest response to beta-blockade since this polymorphism is that associated with the highest response to the adrenergic stimulation in physiologic conditions. By contrary, in their series, authors found the lowest response to the beta-blockade in association with this polymorphism. This contradictory result is not opportunely commented and deserves some cautionary note for the final interpretation of the report.

3-The dose titration of propranolol was achieved in one week for all patients included in the study. This is the most critical point. In real life there is not a fixed period to get the maximum tolerated dose of NSBB. I wonder if further increase of the dose after the one-week period of dose titration would have allowed patients to achieve a higher decrease of variceal pressure.

4-It would be desirable associating the results of the polymorphism to the clinical outcome. This would be extremely original and would give a highest score of interest to the manuscript .

MINOR POINTS The manuscript should be extensively revised for the language. Several typos mistakes can be detected and some sentences are very confusing and should be rephrased.

Responses to Q :

MAJOR POINTS

1- Thank you for your advice. As you suggested, we have added the required details about dose of propranolol in each polymorphism group in the revision. The median daily dose of propranolol was 105±34 mg in the Arg16-Gln27 haplotypes, 113±38 mg in the Gly16-Glu/Gln27 haplotypes, and 108±35 mg in the compound heterozygous, respectively. There were no significant differences across hyplotypes (P>0.05). In addition, we have described the demographic profile of the study population in Tab. 1. No significant differences were found in baseline characteristics and variceal pressure in each group.

2- Thank you very much for correcting for mistakes. As you knew, we have stated that the percentage of the variceal pressure reduction in the Gly16-Glu/Gln27 haplotypes homozygous was significantly greater than that in the Arg16-Gln27 haplotypes homozygous or compound heterozygotes in the text. (22.4±2.1%, 13.1±2.7% and 12.5±3.1%, respectively, P<0.01). Sorry, because of my carelessness, the statements in Table 2 were in contradiction with what in the text, I will correct the mistake carefully and seriously.

3- The plasma half-life of propranolol is 3 to 5 hours. After a period of 3 or 4 half-lives, the serum drug concentration of propranolol will be 85.91% to 93.79 % of the steady state value. According to the literature, the dose titration of propranolol was achieved in one week for these patients included in the study (Gut, Pharmacology of propranolol in patients with cirrhosis and portal hypertension .1985, 26, 14-19). So, after the one-week period of dose titration, increase of the dose would have not allowed patients to achieve a higher decrease of variceal pressure.

4- In this study, we found that the variceal pressure response to propranolol was associated with the polymorphism of the β_2-AR gene. The variceal pressure reduction may be predicted from the polymorphism analysis. Patients with the Gly16-Glu/Gln27 haplotypes homozygote probably benefit from the propranolol therapy.

MINOR POINTS

Thank you very much for your remind. Your suggestions helped us greatly in improving the manuscript. In the revision, a native English speaker from Ponce School of Medicine helped me to revise the unsuitable sentences and typos mistakes carefully and seriously.

Reviewer code: 01221192

Q The paper evaluates the association between the effects of propranolol, variceal pressure and beta-2 adrenergic receptor (β2-AR) gene polymorphism in a group of 64 non-related Chinese cirrhotic patients. The authors found that the variceal pressure response to propranolol was associated with β2-AR gene

polymorphisms, and that the patients with the Gly16-Glu/Gln27 haplotypes seem to benefit more from propranolol therapy. This is an interesting paper, with original data. However, it would be interesting to include in the Discussions a comment on the relation between non-responsivity at propanolol (in terms of portal pressure) and gene-expression profiles. It is well-structured paper, but the English need to be revised (see attached).

Responses to Q : Thank you very much for your comment and language assistant. In previous study, it was found that the HVPG responses to propranolol were similar in different haplotypes homozygous (Influence of beta-2 adrenergic receptor gene polymorphism on the hemodynamic response to propranolol in patients with cirrhosis. Hepatology, 2006; 43 (1): 34-41). According to Laplace's law, variceal pressure is a major determinant of variceal rupture. Moreover, variceal pressure is also a strong predictor of variceal hemorrhage in patients with cirrhosis and noncirrhotic portal hypertension. Therefore, the aim of this study was to evaluate the association between the variceal pressure response to propranolol and β_2-AR gene polymorphism. We found that variceal pressure all decreased after propranolol treatment in each group. The percentage of the variceal pressure reduction in the Gly16-Glu/Gln27 haplotypes homozygous was significantly greater than that in the Arg16-Gln27 haplotypes homozygous or compound heterozygotes.

Reviewer code: 00053417

Q The effect of single nucleotide polymorphisms (SNPs) in the β2-adrenergic receptor (ADRB2) gene on the risk of COPD and lung function,

and on clinical response to drugs in asthmatic patients has been documented. In this manuscript the authors investigated its effect on cirrhotic patients responded to propranolol, which is innovative. There are some reservations about the study, which needs revision:

1- The term "haplotype" is used as a key word in the manuscript. A haplotype is a set of SNPs at a single chromosome or a chromosome pair which are statistically associated. However, there is no description of relevant data in the whole paper. Whether or not the term "homozygote" should be used there?

2- It needs to explain the detail data about the diagnosis of cirrhosis, e.g. the number of patients diagnosed by liver biopsy, clinical data, imaging techniques or presence of the esophageal varices. 3 English needs polishing.

Responses to Q :

1- Thank you very much for your remind. Your suggestions help us greatly in improving the manuscript. In the revision, we will change the term "haplotype" to the term "homozygotes".

2- All the patients were diagnosed as cirrhosis by liver biopsy, clinical data and imaging findings.

3- In the revision, a native English speaker from Ponce School of Medicine helped us to revise the unsuitable sentences and typos mistakes carefully and seriously.

Reviewer code: 00050424

1. The authors do not give data on the dose of propranol that each patient was taking. Were there differences in the mean final propranolol dose in each group ;;

2. They do not describe how frequently they increased the dose. Every other day ;;

3. Were all patients receiving the best propanol dose at 7 days or not ;;; How reliable is the variceal pressure measurement ;; Is there a good correlation between variceal pressure (as it was measured) and portal pressure (HPVG) ;;

4. The authors although they describe how they classified esophageal varices, they do not provide data about the variceal size and other characteristics of the patients.

5. How long before the study somatostatin infusion was stopped ;; How many patients were under somatostatin infusion in each group ;;; Could this medication have influenced the results ;;;

Responses to Q :

1- Thank you for your comment. As you suggested, we have added required the details about dose of propranolol in each polymorphism group in the revision. The median daily dose of propranolol was 105±34 mg in the Arg16-Gln27 haplotypes, 113±38 mg in the Gly16-Glu/Gln27 haplotypes, and 108±35 mg in the compound heterozygous, respectively. There were no significant differences in the mean final propranolol dose in each group ($P>0.05$).

2- In the revision, we will add the details about how frequently the dose was increased in the patients. “After baseline measurement, propranolol was

given orally at an initial dose of 20 mg 3 times daily and was increased by 20 mg every day over a period of 7 days until the resting heart rate had been reduced by 25% or was less than 55 beats per minute".

3- The plasma half-life of propranolol is 3 to 5 hours. After a period of 3 or 4 half-lives, the serum drug concentration of propranolol will be 85.91% to 93.79 % of the steady state value. According to the literature，the best propanol dose was achieved in one week for these patients included in the study (Gut, Pharmacology of propranolol in patients with cirrhosis and portal hypertension .1985, 26, 14-19).

In previous study, variceal pressure measured by this method was found to have a good correlation with HVPG (A computerized endoscopic balloon manometry to detect esophageal variceal pressure. Endoscopy, 2009;46(5):415-20).

4- The aim of this study was to evaluate the association between the variceal pressure response to propranolol and β_2-AR gene polymorphism. We provided data about the variceal pressure and other characteristics of the patients (Tab 1). However, endoscopic variceal findings were not compared for different groups before and after propranolol administration.

5- Thank you for your comment. Somatostatin infusion was stopped 2 hours before starting the variceal pressure measurement. In the revision, we will add this detail about somatostatin infusion. The reduction in portal pressure achieved with the somatostatin bolus lasted less than 5 min due to the short half-life (1-3 min) of somatostatin (The effect of different doses of a bolus injection of somatostatin combined with a slow infusion on transmural esophageal variceal pressure in patients with cirrhosis. J Hepatol 1994;

20(1):27-31.). So, this medication could not influence the results.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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