ANSWERING REVIEWERS

Dear Editor,

Title: The Role of H2S in Portal Hypertension and Esophagogastric Junction Vascular Disease

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

2 H_2S, NO, CO all have biological effects, some of them rather being rather similar. Any claim of an H_2S impact has to control the other gases as well.

Method for measuring apoptosis should be explained more in detail.

Answer: We added the information about the Cell apoptosis assays in the Patients and Methods section. In other publications, controls for other gases than the one which as under investigation have also not been done. So we did not take this issue into consideration when we designed the study, but in principle it should be done.

FACS is mentioned but not in the results?

Answer: we added the FACS information into the result section

The number of measurments should be indicated

Answer: We measured each sample at least 3 times and added this information into the Patients and Methods section.

Presentation of data are presented as mean ± SD only is justified if normal distributed.

$p<0.05$ should be considered statistically significant only if not multiple comparisons are done.

Answer: We followed our suggestion and analyzed the data for normal distribution. No multiple comparison was performed.

The study started with 200 patients, but in table 1 there are left only 23 with portal hypertension?

Answer: We are so sorry that we mis-submitted a wrong table to you, this time we re-submit the new complete table.

Table 1. Comparison of age and plasma H_2S levels between portal hypertension patients and healthy controls.

	Portal Hypertension Group		Control Group	
	n		n	
Age	200	43.6±14.4	100	47.1±12.6
PVD (cm)	200	1.5±0.4*	100	1.1±0.3
H_2S			100	43.5±6.2
Child-Pugh score A	48	42.6±4.7*		
Child-Pugh score B	125	33.5±7.7**▲		
Child-Pugh score C	27	22.2±7.9***▲▲■■		

*Statistically significant different compared with the control group, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

▲ Compared to the Child-Pugh score A group, the Child-Pugh score B and Child-Pugh score C group values were both statistically significant lower, ▲ $p<0.05$, ▲▲ $p<0.01$.

■ Compared to the Child-Pugh score B group, the Child-Pugh score C group had a statistically significant lower value, ■■ $p<0.01$.

What is the concentration of H_2S? Why using so high concentrations in the cell culture?

Answer: We decided by preliminary experiments to use this H_2S concentrations in order to clearly show the effect of H_2S.

Magnification of histological images? Electron microscopy showed cell damage in the controls, but less with low concentration of H_2S?

Answer: the magnification of the histological images was 40X we added this into the figure legends. The damage of the mitochondria was most obvious in cells which were incubated with H_2S. The control without H_2S had intact well shaped mitochondria.

What is low concentration, how many repetitions, what time of culturing? Is there any optimum concentration of H_2S?

Answer: Actually low concentration means no H_2S substitution. When we did the cell cultures we used 3 repetitions and cells were harvested 30min after adding H_2S. We considered the optimum concentration of H_2S the same as the physiological serum concentration of 43.5 = 50 μM.

Correlation betwwen liver damage Child-score and H_2S measurements? any other measurments of liver enzymes as parametr of liver cell damage?

Answer: We added other liver enzyme values of the 200 patients when they were admitted to the hospital into table 1 as parameter of liver cell damage.

	liver cirrhosis	Normal
ALB(g/dl)	33.0±3.7	38.4±4.1
TB(umol/L)	31±24	14±13
ALT(U/L)	37±29	25±26

3 Major concerns 1) Can Authors explain the enormous difference in numbers of patients and controls when comparing the "Material and Methods" (200 patients with cirrhosis-induced portal hypertension and 100 healthy controls, respectively) and the "Results" sections –Table 1- (23 patients and 25 controls, respectively). 2)

Rather than including only cirrhotic patients with portal hypertension and compare them with healthy subjects, it would be better to include also a group of cirrhotics without

portal hypertension. In this way, it could be possible to compare directly cirrhotics with and without portal hypertension, and even to test if H_2S is associated with portal hypertension independently from liver dysfunction.

Answer: We added the complete data sets as new Table 1 to the manuscript. It is difficult to find patients with liver cirrhosis without portal hypertension.

3) In Results, Authors speak about an inverse correlation between H_2S plasma levels and portal diameter. However, they refer to Table 1), where no correlation is shown and only the difference in portal diameter between patients and controls is presented.

Answer: We added a new figure 1, which shows a correlation between H_2S plasma levels and portal diameter ($r=-0.478$, $p<0.05$)

4 References and typesetting were corrected

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

Sincerely yours,

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