ANSWERING REVIEWERS

January 14, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 15531-Reviewed.doc).

Title: Metadoxine improves three and six-month survival rate in patients with severe alcoholic hepatitis: A randomized controlled trial.

Author: Fátima Higuera-de la Tijera, Alfredo I. Servín-Caamaño, Aurora E. Serralde-Zúñiga, Javier Cruz-Herrera, Eduardo Pérez-Torres, Juan M. Abdo-Francis, Francisco Salas-Gordillo José L. Pérez-Hernández.

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 15531

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

1 Format has been updated:
In the manuscript you can see the corrections highlighted in green color.

(q1)Author´s contributions were rewritten according with the suggested format:
Higuera-de la Tijera F designed the study, provided financial support though stimulus "Angeles Espinosa Yglesias 2010", supervised the study, reviewed the statistical methods and wrote de final manuscript; Servín-Caamaño AI and Serralde-Zúñiga AE contributed with the acquisition, analysis and interpretation of data and wrote the manuscript; Cruz-Herrera J, Pérez-Torres E, Abdo-Francis JM and Salas-Gordillo F contributed with the acquisition of data and took care of the patients. Pérez-Hernández JL reviewed the statistical methods and wrote the final manuscript. All authors read and approved the final manuscript.

(q2) We added this paragraph (we sent also the corresponding PDF files).
Ethics approval: The study was reviewed and approved by the "*Hospital General de México, Dr. Eduardo Liceaga*" Institutional Review Board.

(q3) We added this paragraph (we sent also the corresponding PDF file)
Clinical trial registration: This study is registered at **http://clinicaltrials.gov**. The registration identification number is **NCT02161653**

(q4) We added this paragraph (we sent also the corresponding PDF file)
Informed consent: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

(q5) We added this paragraph (we also sent a letter signed by all the authors, as a PDF file)
Conflict-of-interest: The authors have not conflict of interest to declare.

(q6) We added this paragraph:
Data sharing: Technical appendix, statistical code, and dataset available from the corresponding author at **fatimahiguera@yahoo.com.mx**. Consent for data sharing was not obtained but the presented data are anonymized and risk of identification is low. No additional data are available.

(q7) We added the telephone number: +52-55-27892000 **ext.** 1523

(q8) We reformat all the reference numbers as you requested.

For example: Severe alcoholic hepatitis (AH) has a high mortality-rate despite of standard therapy[1,2].

(q9) We added PMID number and DOI to the references.

(q10) we elaborated the section "Comments" as you requested:

COMMENTS

Background

Severe alcoholic hepatitis is a disease with high mortality rate despite of the standard therapy with steroids or pentoxifylline. Oxidative stress plays a key role in the physiopathology of alcoholic hepatitis; therefore, this represents a novel therapeutic target that must be investigated.

Research frontiers

In previous studies has been demonstrated that metadoxine increases the metabolism and depuration of ethanol and acetaldehyde in liver and plasma, and prevents damage caused by ethanol and acetaldehyde in hepatocytes and hepatic stellate cells; it also acts as an antioxidant because the ion-pair molecule is capable of dissociating to N-oxide, which acts as scavenger to trap reactive oxygen species and free radicals. Furthermore, metadoxine can prevents steatosis and injury associated with lipoperoxidation. Metadoxine is a drug currently indicated for treating acute alcohol intoxication; also several studies validate its use for treating alcohol dependence. However, until the current study, metadoxine had never been evaluated as a therapy for patients with severe alcoholic hepatitis.

Innovations and breakthroughs

In the current study we found that metadoxine is an effective therapy for severe alcoholic hepatitis, patients treated with metadoxine had better survival at 3 and at 6 months compared with those treated with standard therapy with steroids or pentoxifylline. Furthermore, it is well known that alcohol abstinence is an important factor associated with long-term survival in these patients. In this study we found that patients who received metadoxine were more able to maintain alcohol abstinence, this fact could be related to the improve in six-month survival in patients treated with this drug.

Applications

The study results suggest that metadoxine could be used as an effective therapy for those patients with severe alcoholic hepatitis, and validate results of other previous studies that have found that metadoxine is

an effective therapy to achieve alcohol abstinence.

Terminology

Severe alcoholic hepatitis is a condition characterized by a rapid onset of jaundice in the absence of biliary tract obstruction, painful hepatomegaly and ascites, transaminases $\geq$ two times above the normal value, aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio $\geq$ 2, neutrophilia, total bilirubin > 5 mg/dL, and Maddrey´s discriminant function (MDF) >32 (calculated with the formula [4.6 x (patient prothrombin time (PT) – control PT, in seconds) + total bilirubin in mg/dL]), which occurs in patients with a history of chronic and heavy alcohol intake.

Metadoxine is the ion par between pyridoxine and pyrrolidone carboxylate, the cyclic amid of glutamic acid, responsible for the synthesis and catalization of glutathione.

Peer review

This is an interesting study focusing in a largely unmet need therapeutic area. This is a single-center open label clinical trial comparing pentoxifylline, prednisone or metadoxine alone or in combination to assess their efficacy in severe alcoholic hepatitis. Results showed that groups receiving metadoxine achieved better survival. As in other studies, maintenance of alcohol abstinence was the best predictor of survival. This study found that alcohol abstinence is an independent prognostic factor in the six-month mortality, and that patients treated with metadoxine were more able to prevent relapse in alcohol consumption. However, intervention did not prevent complications associated to cirrhosis which may be explained either because the study was underpowered or that the effect of abstinence it-self rather than intervention was the main predictor for survival.

2 Revision has been made according to the suggestions of the reviewers.

(Reviewer 34635)

We explained how we calculated the sample size:

Sample size calculation: We employed the statistic program Epidat 3.1. We calculated the sample size considering a difference in the survival rate at 3 months of 30% between groups receiving MTD and those receiving the standard treatment, we also considered a level of confidence of 95% at two-tails, a potency of 80%, and an additional 20% of subjects considering potential losses, we obtained a total of 35 patients per group.

We explained how we made the randomization.

Randomization: A total of 217 patients were evaluated, of them 135 patients were diagnosed with clinical and biochemical criteria for SAH and met the inclusion criteria. These patients were randomized into four treatment groups. For randomization we employed the statistic program Epidat 3.1 for constructed a table of random numbers, considering four groups of equal size.

In the discussion we provided the explanation about how metadoxine helps in alcohol abstinence.

In our study, relapse in alcohol intake was the main independent predictor factor of mortality at 6 months. Alcohol abstinence is considered the cornerstone in the management of alcoholic hepatitis (AH). Wang T[43] demonstrated that alcohol abstinence ameliorated AH by decreasing the liver enzyme and fibrotic markers, and improving hepatic steatosis. In our study, therapy with MTD helped patients to maintain alcohol abstinence. This finding is similar to those reported by several studies that have demonstrated that MTD is an effective therapy for abstinence[44-48]. Currently, disulfiram, naltrexone and acamprosate are approved

for the treatment of alcoholic dependence; however, all these medications are contraindicated in patients with severe liver disease[44], such as our patients. Patients who have recovered from an episode of severe alcoholic hepatitis must be helped to maintain alcohol abstinence without risk or compromise for your liver function. Guerrini I[48] found that alcoholic patients who received treatment with MTD achieved alcohol abstinence in a greater proportion compared with those who did not receive it. More recently, and interesting retrospective analysis by Leggio L[44] found that patients with ALD who were treated with MTD had a significant decreased in drinks per week, likewise improvement in AST/ALT ratio, compared with those who did not receive it. Neurological benefit effects of MTD therapy in patients with attention-deficit/hyperactivity disorder have been demonstrated in several studies conducted by Manor I[49-51]. In animal models, the effects of MTD on CNS have been studied. Ethanol and acetaldehyde increase the activity of dopamine neurons in reward areas of the CNS, these actions are associated with the rewarding and reinforcing properties of the ethanol. MTD could favors abstinence through its ability to metabolize and to clearance ethanol and its metabolites from the organism, but also through its direct effect on neurotransmitters, such as, gamma-aminobutyric acid, acetylcholine and dopamine, all of them involved in the neurobiology of alcohol craving[44-51].

We discussed the new interesting results from the STOPAH trial.
The investigators from the "*Steroids or Pentoxifylline for Alcoholic Hepatitis*" (STOPAH) trial[23], recently presented at the Liver Meeting 2014, which takes place in Boston, Massachusetts. The STOPAH trial was a multicenter, double-blind, factorial (2x2) trial, which included 1103 patients who were randomized to one of four groups: prednisolone + placebo, PTX + placebo, prednisolone + PTX, or double placebo. The investigators found that prednisolone, but not PTX, was associated with a lower risk for 28-day mortality. In contrast, the mortality rate in the group who received PTX was similar to those who received the double placebo. Beyond 28 days, neither drug was associated with a survival benefit, and infections were about twice as frequent in the prednisolone group.
In our study, relapse in alcohol intake was the main independent predictor factor of mortality at 6 months. Alcohol abstinence is considered the cornerstone in the management of AH. In the results from STOPAH trial[23], relapse in alcohol consumption had a deleterious effect; at 1 year, patients who did not cut down or who increased their alcohol consumption had a 3-fold risk for death, compared with patients who abstained (OR, 2.99; $P < .001$). Patients who reduced their drinking, but not below safety limits still had a more than a 2-fold risk for death at 1 year, compared with patients who abstained (OR, 2.28; $P = .032$), as did patients who reduced their drinking to below safety levels (OR, 2.17; $P = .031$).

(Reviewer 2936306)
Acamprosate is a drug approved for maintain alcohol abstinence; however, it is contraindicated in patients with severe liver disease, such as our patients, for this reason we did not use it in patients with severe alcoholic hepatitis. To answer your ask about why we did not use Acamprosate in our patients we added a paragraph in our discussion section:

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(Reviewer 185965)

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We suppressed the abbreviation **SAH** according with your kindly suggestion.

3 References and typesetting were corrected

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

Sincerely yours,

Fátima HIGUERA-DE LA TIJERA, MD, MSc.
Liver Clinic, Gastroenterology Department
Mexico´s General Hospital "Dr. Eduardo Liceaga"
Mexico City, Mexico
Tel. +52-55-27892000 ext. 1523
E-mail: fatimahiguera@yahoo.com.mx