Format for ANSWERING REVIEWERS

July 23, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 20709-Review.doc).

Title: Role of pentoxifylline in non-alcoholic fatty liver disease in high-fat diet- induced obesity in mice

Author: Simone Coghetto Acedo, Cintia Rabelo e Paiva Caria, Érica Martins Ferreira Gotardo, José Aires Pereira, José Pedrazzoli, Marcelo Lima Ribeiro, Alessandra Gambero

Name of Journal: *World Journal of Hepatology*

ESPS Manuscript NO: 20709

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

Reviewed by 02861012

Manuscript Number	20709
Manuscript Title	THE ROLE OF PENTOXIFYLLINE IN NON-ALCOHOLIC FATTY LIVER DISEASE IN HIGH-FAT DIET- INDUCED OBESITY IN MICE.
Review Time	2015-06-23 19:15

The authors studied the effects of pentoxifylline in an obesity model in order to understand its potential effects in NAFLD. However, there are several points that are unclear and need to be addressed:

1. The authors do not state the actual number of animals used per group.

It was included in Material and Methods section.

2. As stated in introduction, NAFLD occurs more often in males and primarily affects the middle aged and the elderly; however here the authors used mice that were 4-week old, why?

Mice were acclimatized in our Animal house during 2 weeks before the introduction of experimental diets. Ten weeks after, pentoxifylline treatment was performed. Thus, animals are 18 weeks old at the end of protocol. This is the current obesity protocol used in many studies.

3. In the current study mice were treated with HFD for 12 weeks and the last two weeks they received pentroxifylline. In the other studies they describe in discussion and compare their results, pentoxifylline was administered for more than 2 weeks. It is unclear where the authors based their protocol?

This treatment protocol was based in previous works from our research group, where only two weeks of treatment was able to show liver or adipose tissue improvements in this experimental model (The immunosuppressant drug, thalidomide, improves hepatic alterations induced by a high-fat diet in mice. Pinto Lde F, Compri CM, Fornari JV, Bartchewsky W, Cintra DE, Trevisan M, Carvalho Pde O, Ribeiro ML, Velloso LA, Saad MJ, Pedrazzoli J Jr, Gambero A. Liver Int. 2010 Apr;30(4):603-10; Effects of methotrexate on inflammatory alterations induced by obesity: an in vivo and in vitro study. DeOliveira CC, Acedo SC, Gotardo EM, Carvalho Pde O, Rocha T, Pedrazzoli J Jr, Gambero A. Mol Cell Endocrinol. 2012 Sep 25;361(1-2):92-8.)

4. The effect of pentoxifylline in inflammatory cell infiltrate should have also been studied.

Cell infiltration was evaluated during liver histology analysis, as presence or absence of inflammation.

5. Minor comment: Figure 1 labelling is not clear.

It was modified.

Reviewed by 00502973

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Review Time	2015-06-22 20:23

In the current manuscript, Acedo et al reported that administration of pentoxifylline was able to reduce the fat accumulation in liver of obese mice fed by high-fat diet. This study is helpful to better understand the mechanism of pentoxifylline on NAFLD. English could be improved though it is acceptable at current status. Concerns exist and should be addressed. Major concern: As pentoxifylline is considered as an antioxidant, its effects on oxidative injury may be involved in its effects on fatty liver. Oxidative stress is also involved in the pathogenesis of steatohepatitis. Therefore, the effects of pentoxifylline on oxidative injury should be involved in current study such as on lipid oxidation.

We added this hypothesis in discussion with a reference, but it did not help us to explain the results obtained with lean mice.

Minor concerns: 1. Introduction: The author stated “NAFLD diagnosis requires the exclusionof secondary etiologies, including alcohol consumption, hepatitis C and drug usage ”. HBV infection should also be excluded before the diagnosis of NAFLD especially in Asia.

We included in Introduction section.

2. Necropsy and sample collection: The author stated “Liver and adipose tissue was perfused with 15 mL Phosphate Buffered Saline (PBS), collected and weighed.” The author should clarify where the adipose tissue was taken from.

We apologize for this mistake. Only liver was perfused ex vivo. It was corrected in Material and Methods section.