Response to Decision letter for WJG-45986

Dear editor Ma：

Thanks for giving me the chance to modify my manuscript entitled "Proteomics of mediodorsal thalamic nucleus in rats subjected to restraint water-immersion stress". We remodified our manuscript according to reviewers' comments, 45986-CrossCheck report, and 45986-Edited (download).

Our point-by-point responses to the reviewers' comments are provided below.

Reviewer #1:

Thank you for your recognition of our work.

1. Authors claim that "the identification of, targets for more specific therapies". It is unclear to which disease they refer their willingness to find more specific therapies.

Reply: Stress-induced gastric mucosal lesion (SGML) is one of the most common visceral complications after trauma. Restraint water-immersion stress (RWIS) is a kind of heavy-duty compound stress that can cause serious gastrointestinal dysfunction and has been widely used to study the pathogenesis of SGML to identify medications that can cure the disease.

2. "Gastric mucosal lesions were identified with a magnifying lens and measured using the erosion index (EI)". Reference 42 does not mention any magnifying lens or index (EI). Therefore, how the result statement: "The damage index was significantly different between the control and RWIS groups" raises problems of understanding regarding the evaluation method.

R**eply:** Thank you and I'm sorry to such a mistake. We reviewed Guth's documents and found that it did not mention any magnifying lens or index (EI) in the reference 42. The scored method of gastric mucosal lesions was described in Guth's document in 1979 not 1992. I have been replaced reference 42 with the literature 'Guth PH, Aures D, Panlsen G. Topical aspirin plus HCl gastric lesions in the rat. Cytoprotective effect of prostaglandin, cimetidine, and probanthine. Gastroenterology, 1979, 76(1):88-93.'.

3. The Results Section "Identification of differentially expressed proteins" reports not only the results of the study, but also hints of methodology and even principles for the interpretation of the results. In reality, concepts other than pure results should be included in other sections of the manuscript.

Reply: We greatly appreciate your valuable advice. The physiological response to stress is complex, and many metabolic pathways and proteins are expressed differently during Restraint water-immersion stress (RWIS). When combined with mass spectrometry (MS) and bioinformatic approaches, iTRAQ becomes a powerful tool to explore the response in proteins to stress. We compared the differentially expressed proteins in the MD using iTRAQ technology to provide more complete and comprehensive information on the molecular mechanism of the MD in rats subjected to RWIS. The results of the proteomic analysis were verified by Western blotting. These data address the original aim of this study, but the functions of key proteins linked to gastric ulcer, eg GSK3B, during RWIS should later be verified by qrt-pCR or immunoblotting, and further functional studies using RNAi. We take it into account in further study. Thank you again.

4. A clear message of translational medicine is lacking.

R**eply**: To screen potential curative drugs of stress-induced gastric mucosal lesion (SGML) has created an urgent need to identify new targets. We compared the differentially expressed proteins in the MD using iTRAQ technology and identified 65 dysregulated proteins and analysed their subcellular localizations, molecular functions, biological processes and signalling pathways. But physiological response to stress is complex, and many metabolic pathways and proteins are expressed differently during Restraint water-immersion stress (RWIS). Development of more reliable management strategies depend on accurate biological information with respect to the impact of dysregulated proteins on SGML. The functions of key proteins linked to gastric ulcer, eg GSK3B, during RWIS should later be verified by qrt-pCR or immunoblotting, and further functional studies using RNAi. We take it into account in further study. Thank you again.

Reviewer 2:

Thank you for your recognition of our work.

1. Research methods and results appeared to be well performed and interesting. Downregulation of GSK3B is intriguing and how is this linked to gastric ulcer deserve further investigation. In the current paper, it is asham that authors only validated the expression level of GSK3B but did not further examine serine 9 and tyrosine 216 phosphorylations of GSK3 or any of its downstream substrates. If author provide those results, this paper could further correlate the expression data with actual GSK3 "enzyme activity". The impact of the paper will be further elevated.

Reply: The physiological response to stress is complex, and many metabolic pathways and proteins are expressed differently during Restraint water-immersion stress (RWIS). When combined with mass spectrometry (MS) and bioinformatic approaches, iTRAQ becomes a powerful tool to explore the response in proteins to stress. We compared the differentially expressed proteins in the MD using iTRAQ technology to provide more complete and comprehensive information on the molecular mechanism of the MD in rats subjected to RWIS. We identified 65 dysregulated proteins and analysed their subcellular localizations, molecular functions, biological processes and signalling pathways. The results of the proteomic analysis were verified by Western blotting. These data address the original aim of this study.

We greatly appreciate your valuable advice. The functions of key proteins linked to gastric ulcer, eg GSK3B, during RWIS should later be verified by qrt-pCR or immunoblotting, and further functional studies using RNAi. We take it into account in further study. Thank you again.

2. In addition, previous study shown that Restraint water-immersion stress (RWIS), similar to insulin, can affect glucose uptake in brain and gastric secretion. Addition of a discussion about the potential role of GSK3 in this pathway is required.

Reply: Thank you for your valuable advice. Glycogen synthase kinase 3 (GSK- 3) is

commonly found in mammalian eukaryotic cells, which not only exists in the cytoplasm but also exists in some of the cell area (such as the nucleus, mitochondria). GSK-3B is so widespread in the central nervous system, mainly in neurons, while glial cells are also expressed. GSK-3B is negative regulatory factor in glucose homeostasis, involved in energy metabolism, inflammation, and endoplasmic reticulum stress, mitochondrial dysfunction and apoptosis pathways, and so on. In recent years, results indicate that GSK-3B is involved in many signaling pathways, which related to the nerve protection, neuron axon length adjustment, cognition, learning and memory, and a variety of neurodegenerative diseases and so on. But the regulation function of GSK-3B in gastric stress remains to be further studied.

We greatly appreciate your valuable advice. We discussed the potential role of GSK3 in this pathway furtherly in the part of discussion. Thank you again.

Best regards

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