

Point-by-point Response (Manuscript NO. 59582)

Reviewer #1:

Specific Comments to Authors: The manuscript describes protective effects of intestinal trefoil factor on stress induced-gastric pathological changes. Although all results are predictable and there is no estimable scientific significance, the contents will be acceptable for publication in WJG if construction and writing are improved as suggested in attached file.

Response: Thank you for your careful work, we really appreciate it. We have revised our manuscript according to your kind suggestions one by one in the attached file. The current version got a grade A in language evaluation after polishing by native speakers. Here is the brief summary of our revision, you can check the details (highlighted) in the revised version of manuscript.

Abstract:

- (1) “too many critical”: “a critical issue” was changed to “an unsolved issue”; “routine critical care” was changed to “routine intensive care”;
- (2) “GSE-1 cell (explain)”: human gastric epithelium cell line;
- (3) The “Results” has been rephrased to make it more specific in the revised version.

Introduction:

“NSAIDS (explain)”: non-steroidal anti-inflammatory drugs.

Methods and Materials

- (1) “Brief explanation of WIRS, also add the method of sacrifice”: Some relevant content has been added. “WIRS”: “Briefly, in the stress exposure session, each rat was restrained individually in a plastic cage and immersed up to its xiphoid in temperature-controlled water (23°C) for 16h.”; “method of sacrifice”: All the rats were anesthetized with intraperitoneal sodium pentobarbital (50mg/kg, Sigma-Aldrich, MO, USA) and sacrificed by cervical dislocation;
- (2) “sparate as Experimental design” and “WIRS+ITF+LY group (define)”: We have added the “Experimental design ” section, and each group of rats has been defined in the revised manuscript.

- (3) “cell culture flasks (Add size, Cat. No. manufacturer)”: cell culture flasks (25cm², Cat. No. 430639, Corning, NY, USA);
- (4) “Fetal Bovine Serum” was changed to “fetal bovine serum”;
- (5) Some sentences involving cell culture and cell viability have been combined and rephrased according to your suggestions;
- (6) “LPS (define)”: lipopolysaccharides (LPS);
- (7) Cat. No. and Manufacturer of CCK-8 and Transwell chambers, and the Cat. No. of confocal fluorescence microscope have been added;
- (8) The “blocking buffer” and “TBST” have been defined;
- (9) “ECL (define)”: enhanced chemiluminescence (ECL) system. By the way, the ECL was used for protein band visualization and the Odyssey Scanning System was used for image capture;
- (10) “FDA/PI staining (define)”: fluorescein diacetate (FDA) and propidium iodide (PI) staining.

Results

We have rephrased relevant paragraphs in line with your kind suggestions to make “Results” more specific and straightforward.

Reviewer #2:

Specific Comments to Authors: In this study, the authors investigated anti-inflammatory profiles of intestinal trefoil factor (trefoil factor 3) in water immersion stress-induced rat gastric ulcer model, and possibly those anti-ulcer profiles of the agents depend on Akt pathways. Although experiments were designed and performed well and the manuscript was written relatively in a straightforward manner, there are minor points to be considered/described by authors, as follows.

1. In Figures 1 and 4, the authors dosed intestinal trefoil factor in rat gastric ulcer model (0.1 mg/kg for 3 days prophylactically). Prophylactic dosing itself is reasonable for this model; however, there is no background data to optimize the dosing regimen of the factor (the dosage and the duration of dosing). The authors have to discuss how they determined the intervention protocol in this ulcer model.

Response: Thank you for your comments. As you mentioned above, for this model, prophylactic dosing itself is reasonable. There is also a reference supporting this dose for rodents (Neuropsychopharmacology (2012) 37, 2671–2683. DOI:10.1038/npp.2012.131). In addition, the dose of 0.01mg/kg and 1mg/kg were also considered, but the former (0.01mg/kg) did not present protective effects and the latter (1mg/kg) did not present better protective effects than the optimal dose (0.1mg/kg).

2. As the authors pointed in the introduction section, ITF itself may promote malignant progression via Akt signaling and it could lead to the limitation of the clinical application of it among ulcer patients. The authors should discuss the potential clinical advantages of ITF over conventional anti-ulcer agents such as PPIs and H2 receptor antagonists having a long clinical history and relatively validated safety clinical profiles.

Response: Thank you for your comments. Although expression of ITF has been detected to be elevated or promote progression in some types of cancers, it is still difficult to determine whether ITF is an initiating or driving factor of tumor. By the way, the Akt signaling is associated with inhibiting cell apoptosis and stimulating cell

proliferation, which involve carcinogenesis, tissue damage and repair, etc. As an endogenous peptide, ITF harbors the innate advantage over conventional antiulcer agents (PPIs and H2RAs), leaving the potential side effects to be investigated in the future. In addition, both PPIs and H2RAs might increase the absolute risk of pneumonia, the increased risks of myocardial ischemia, *Clostridium difficile* enteritis have also been reported over the past years. Therefore, it is reasonable to assume that ITF could be a new choice of antiulcer agents. Relevant content has been added and highlighted in the Discussion section in revised manuscript.