Reviewer 1: Major revision

Strength: Looked at effect of H2 antagonists on efficacy of 5FU in addition to its effect on undesirable effects of the chemotherapy. Also, made some investigation into the mechanism underlying the protective effects of H2 antagonists. Used multiple H2 antagonists with different mechanisms of action allowing for distinction between classes that produce protective effects and classes that do not.

Addresses a real clinical problem and limitation to therapy and attempts to gain mechanistic insight in how the clinical problem (chemotherapy induced intestinal mucositis) can be reduced/managed. In examining a mechanism, have identified a new mechanism as to how such drugs can produce protective effects. This may allow for production of new targeted therapies.

**This paper needs some rewriting. Some of the sentence structure is awkward and ambiguous. Reader is left to read between the lines at times and make assumptions regarding what the authors' point is. In the interest of being concise and limiting pages, some important content is missing.**

***the introduction could be expanded to include more detail of current literature in the area and more specifics with regards to their own past studies. They have them referenced but if using them as the rational for the present studies, there should be more detail provided here.***

***abstract is misleading.......Results: "lafutidine,...inhibitied the 5-FU induced side effects, including weight gain, mucosal damage, and mucin accumulation. Written this way makes is sound as though 5FU caused weigh gain and mucin accumulation. This is an example of why rewriting certain sections is necessary so that the real point isn't misconstrued. The author means that Lafutidine prevents 5FU induced weight loss etc....

The aim section of the abstract is poorly written.
"although H2 receptor antagonists appear clinically beneficial???? clinically beneficial in what context? grammar here in the first sentence also needs to be addressed....although..........;however, ...... Not proper sentence structure.

Why two groups of rats? Why not take the intestinal tissues from the Yoshida rats? If there was a reason for the two groups, it should be explained. Also, it isn't clear in the results which group of rats is being used for which analysis. Had to read between the lines here. Are the Wistar rats, tumor bearing? Or just receiving drugs? Also not clear.

There is a lot of detail about specific experimental analysis, that I think could be shortened and references to other articles where the methods are explained in detail could be used. This would allow
for more space to expand the introduction, and experimental design/set up areas that are presently lacking detail.

Reviewer 2: Major revision

Fluorouracile (5-FU) is a drug widely used on solid tumors. It has been demonstrated that the inhibition of DNA synthesis induced by 5-FU caused damage of gastrointestinal tract mucosa and changes in the intestinal mucosal morphology.

In this study the authors investigated the role of H2-receptor antagonists on intestinal mucosal injury induced by 5-FU in rat.

Comments:
The authors have recently published a paper in which the efficacy of H2-receptor antagonists including lafutidine against cisplatin-induced mucositis was evaluated (Ichikawa et al., 2013). The novelty of the present study should reside on the protective role of the H2-receptor antagonists on mucositis induced by another anti-cancer drug, 5-FU.

The authors demonstrate that lafutidine was able to inhibit 5-FU-induced mucosal damage indicating a protective effect against intestinal damage.

However no meccanism underlying the role of H2-receptor antagonist lafutidine in drug-induced intestinal mucosal injury has been investigated.

1) In the figure 2 the quantitative assessment to estimate the length/width ratio of villous in jejunum should be shown.

It would be interesting to evaluate the effect of H2-receptor antagonists on the alkaline phosphatase which is a representative digestive enzyme localized on the mucosal surface.

The authors should evaluate the biosynthetic activity of mucin or the influence of H2-receptor antagonists on diamine oxidase (DAO) activity that is associated with the maturation and the integrity of small intestinal mucosa.

2) Apoptosis is considered to play a crucial role in the occurrence of intestinal mucositis caused by 5-FU chemotherapy. The authors should investigate the role of H2-receptor antagonists on the apoptotic response to 5-FU.

3) The results obtained by the authors indicated that the protective effects of lafutidine involved the function of capsaicin-sensetive sensory neurons. The observed effects could be the activation of capsaicin sensitive calcitonin gene related peptide (CGRP) which produces nitric oxide (NO) in endothelial cells. The authors should evaluate the effects of lafutidine on capsaicin-induced NO production.
Reviewer 3: Revision

Authors described the effect of histamine receptor antagonists on intestinal mucositis induced by 5-FU and on 5-FU antitumor activity in rats. They also addressed the role of capsaicin-sensitive afferent neurons.

Authors showed that one of the tested drug – lutfidine has the positive effect on the intestinal mucositis after 5-FU treatment and only non-significant negative effect on the antitumor activity of 5-FU.

The presented article seems to be sufficiently novel and interesting to warrant publication.

Independently of the significance of the presented data and its quality, some information is missing and some text changes should be done for better readability (see the Comments to authors).

Comments to authors:

1. The Abstract section, AIM: Remove “however” from the first sentence.

2. The Abstract section, AIM: Replace “Furthermore” e.g. by “Simultaneously” in the third sentence.

3. The Abstract section, METHODS: It is not clear what concentration of 5-FU was used in experiments with combination of 5-FU with H2 receptor antagonists. This information is not clear even from the Method section.

4. The Abstract section, RESULTS: Remove “Moreover” from the second sentence.

5. The Introduction section: Remove “Therefore” from the sentence: “Therefore, these findings suggest....”

6. The Introduction section: Correct the sentence: “The clinical practice guidelines recommend using either a proton-pump inhibitor (omeprazole) or an H2-receptor antagonist (ranitidine) for the prophylaxis of epigastric pain after treatment with cyclophosphamide, methotrexate, and 5-FU alone or in combination with folinic acid [8]. Instead of “5-FU alone or in combination with folinic acid” use „5-FU or with combination of 5-FU and folinic acid“.

7. The Introduction section: Include the information about capsaicin-sensitive sensory neurons, as it is not clear why the experiments with capsaicin were done.

8. The Material and Methods section: The clear information about the number of rats used and what rats were used for implantation of sarcoma cells is missing. The passage about rats and their treatment has to be rewritten accordingly.

9. The Material and Methods section: The information about the number of Wistar rats used in particular experiments is missing.
10. The Material and Methods section: Add the information about the supplier of the anti-mucin antibody.

11. The Material and Methods section: Remove the word “all” in the sentence “Regarding PGM34, it was recently demonstrated that the epitope of this monoclonal antibody was a specific sulfated oligosaccharide of the mucin molecule; the antibody stains all the goblet cells of the small intestine of the rat [13].” (page 5, MM), as the cited authors did not write that this antibody stains all goblet cells but most of them.

12. The Material and Methods section: Correct the centrifugation speed for 8,000 x g (page 5, MM).

13. The Material and Methods section: Part 2.7 Statistical analysis: only Tukey’s test is mentioned. However, in the description of the table 1 authors mentioned Dunnett’s test. Include description of this test and clearly state in which experiments which one of the tests was used.

14. The Result section, page 6: Bodyweight change: In the Figure 1 it is apparent, that authors weighted rats also on 2nd, 4th and 6th day. This information is missing in the Material and Methods section.

15. The Result section: Figure 1. Famotidine and cimetidine is related to the control group and lafutidine to 5-FU-treated group. It should be changed and/or explained.

16. The Result section: From the graph in Figure 1 it is not apparent that cimetidine caused virtually no changes in the weights of rats as compared to 5-FU treated group. After 8-days the BW change was around 15% comparing to 5-FU treated group. Does it mean non-significant change?

17. The References section: reference 11 – remove the dot at the end of the reference; reference 22 - remove the “x” and dot at the end of the reference.

Reviewer 4: Major Revision

In the manuscript from Ichikawa et al, entitled "Influence of H2-receptor antagonists on intestinal mucositis induced by 5-fluorouracil in rats", the authors compared the influences of cimetidine, famotidine, and lafutidine on intestinal mucositis induced by the antitumor 5-fluorouracil (5-FU) in rats. They also analyzed the efficacy of these H2-receptor antagonists against 5-FU induced rat intestinal mucosal injury and evaluated their effects on mucin accumulation in different areas of the intestinal tract. This is an interesting research question, however there are some major concerns regarding the experiments performed and data presentation that need to be addressed by the authors, as pointed out below:

1 – There is no explanation in the manuscript about the characteristics of the Yoshida sarcoma and why it was used in the present work.

2 – For the immunohistochemistry, there is no information about the anti-mucin monoclonal antibody PGM34: from which company/researcher it was obtained, which dilution was used.
3 - Fig 2. Scale bars should be included in the images, as “magnification 25X” is not a precise way to describe the final magnification of an image.

4 – Fig. 2 - images are very poor in contrast and resolution and therefore they need to be changed/improved.

5 – Fig 2. Legend says “Note that goblet cells in the jejunum and ileum show positive staining with PGM34” but since the images are very poor in resolution it is not evident where is the PGM34 positive staining. Arrows are needed.

6 – Image H in Figure 2 (5-FU + cimetidine) is the same as image Figure 3B (5-FU in capsaicin pretreatment). How the same image is shown for two different treatments?

7 – Since 5-FU is known to cause the degeneration of the basal epithelial cells in the intestinal mucosa, one important missing experiment is the analysis by immunohistochemistry of the structural integrity of the basal epithelial layer using antibodies against: specific keratin molecules, tight junction proteins (occludins, claudins and ZO5), and apoptosis markers (activated caspase 3). Valuable information could come from these analysis in all the experimental conditions: control, 5-FU, and 5-FU + cimetidine, famotidine, and lafutidine.

(end)