

Manuscript ID: 108375

Manuscript Title: Ulcerative colitis: Timeline to a Cure

Answers to questions, Reviewer # 1:

I would like to begin by sincerely thanking the author for her valuable and thought-provoking contribution to the field of inflammatory bowel disease (IBD). This manuscript presents a bold and compelling argument that challenges the dominant immune-dysregulation paradigm in ulcerative colitis (UC) and proposes a novel redox-based etiology. The thorough synthesis of historical, clinical, and biochemical evidence reflects a deep understanding of the topic and a courageous scientific vision. It is a privilege to review such a work that critiques the status quo and offers a potential path toward curative therapy. Before proceeding to its publication, I have some concerns/suggestions that must be addressed in the revised manuscript. These comments are as follows;

I sincerely appreciate the Reviewer's kind comments and thoughtful suggestions. All the recommendation will have a positive impact on the overall readability and comprehension of my manuscript. I have endeavored to answer all questions and make the modifications brought forth by the Reviewer below.

- 1. briefly stating the type of evidence used (e.g., clinical observations, biochemical rationale, therapeutic trials).** Thank you for pointing this out. I have added the following sentence to the first paragraph of the conclusion: *"This is supported by cessation of rectal bleeding within 1-2 weeks after initiation of treatment in addition to colonic biopsy results showing histologic remission in a case series of 36 patients with refractory UC"*.
- 2. Specify that the therapeutic remission described is based on reducing agents—many readers may not be familiar with this approach.** I had already mention this in the second sentence of the conclusion but I added the following sentence to emphasize the point since, as the reviewer mentions, some readers may not have noticed. *"The use of reducing agents to induce and maintain remission in UC is based on the evidence indicating that H₂O₂ plays a causal role in the pathogenesis (development) and pathophysiology (mucosal inflammation) of this inflammatory bowel disease."*
- 3. Clarify the novelty of the redox hypothesis upfront—state how this diverges from prevailing views.** I added a new penultimate paragraph to the introduction, which contrasts the mechanistic functionality and therapeutic utility between the prevailing view of immune dysregulation and an H₂O₂ redox based pathogenesis. This was an excellent suggestion.
- 4. Consider organizing the section under subheadings (e.g., "H₂O₂ generation", "Mitochondrial impairment", "Loss of redox homeostasis") to guide the reader.** Great suggestion. I added 4 new subheadings to the discussion section and a new after the introduction to improve clarity.

- 5. Include quantitative outcomes or patient case studies where reducing agents achieved remission (if available).** I added the reference (in the first paragraph of the conclusion) to a case report showing a normal 2019 colonoscopy and biopsy in a patient with a 30+ year history of refractory UC who was treated in 2007. He is asymptomatic to this day, 18 years later. As far as medical science can determine he is permanently cured of his UC.
- 6. Elaborate on what specific reducing agents were used and their safety profiles.** I added a second paragraph to the conclusion explaining this.
- 7. While maintaining a strong stance, softening some language to meet scientific tone standards (e.g., “crimes against humanity” could be rephrased as “may raise serious ethical concerns warranting further investigation”).** I have re-phrased this as a serious ethical concern.
- 8. long paragraphs into smaller thematic chunks to improve readability.** Thank you for this feedback. I split up a long sentence in the 2nd paragraph of the introduction. I also split up the last sentence in the 1949, 1960 and 1994 and 2001 paragraphs to improve readability. I rephrased the new paragraphs I added in to the manuscript to reduce long phrases. Additionally, I rephrased the last long paragraph of the of the timeline into shorter sentences for improved clarity.
- 9. visual comparison (e.g., bar graph or flow diagram) showing clinical outcomes of immunosuppressive therapies vs. redox therapies could further support the argument.** This would be an effective method for a head to head comparison when formal clinical studies are conducted. Unfortunately, until clinical studies are undertaken I don't have the data for this type of diagram.
- 10. Watch for emotionally charged language that might weaken scientific neutrality:** Thank you for this observation. I will recheck for this and make appropriate changes as needed.
- 11. E.g., “clerical adjustments” → perhaps reword as “methodological modifications” or “trial design optimizations”.** Excellent suggestion. I have added both descriptions into the text.
- 12. “Crimes against humanity” → see note above.** Changes made, see above.
- 13. Ensure consistency in terminology (e.g., “UC patients”, “patients with UC”; “redox homeostasis”, “oxidative imbalance”).** Thank you for this observation. I changed several instances of 'UC patients' to 'patients with UC'
- 14. Minor typographical issue: There are two items marked “7)” in the list of trial design strategies. Renumber accordingly.** Thank you for pointing this out. I have made the appropriate corrections.

15. Include data or published studies supporting the efficacy of reducing agents in achieving histologic remission. I have cited the two case reports that demonstrate the therapeutic effect of reducing agents in ulcerative colitis.

16. Cite studies measuring oxidative stress in UC colonic tissues to support the redox hypothesis.

The definitive study demonstrating significantly elevated colonic epithelial H₂O₂ levels, indicative of increased oxidative stress, was published in *GUT* in 2007, see below (Santhanam, cited in my manuscript). It reported markedly elevated H₂O₂ production in the non-involved ascending colonic epithelium of UC patients in remission, with positive (Crohn's disease) and negative (healthy individuals) controls showing normal H₂O₂ levels, confirming its specificity to UC rather than general inflammation. The authors of the study concluded that *"...the initial event in ulcerative colitis is an increased generation of H₂O₂ from mitochondria. If this were to happen primarily within epithelial cells, it is then possible to attribute all pathogenic events subsequent to this."*

This study demonstrates that colonocytes enhance their redox buffering (antioxidant) defenses to counter increasing H₂O₂ levels and preserve redox homeostasis. However, excessive H₂O₂ production overwhelms this capacity, resulting in intracellular accumulation and extracellular diffusion, which triggers neutrophil recruitment leading to mucosal inflammation and ulcerative colitis.

The pre-inflammatory rise in colonocyte H₂O₂ is localized to colonic epithelial cells and remains undetectable via colonoscopy or histological analysis. Upon extracellular diffusion, H₂O₂ recruits neutrophils to the colonic epithelium, exacerbating oxidative stress through significant H₂O₂ release, leading to tissue damage, inflammation, and ulcerative colitis. Immunosuppressive agents mitigate neutrophil-driven inflammation but fail to address the persistent H₂O₂ buildup in epithelial cells, which resumes extracellular diffusion upon treatment cessation. This underlying mechanism explains UC's relapsing nature and the non-curative effect of immunosuppressive therapies.

This study experimentally confirmed my earlier prediction that H₂O₂ disrupts beta-oxidation by inhibiting mitochondrial thiolase, a crucial enzyme in the beta-oxidation pathway. The cause of impaired colonic epithelial beta oxidation preceding relapse was a mystery since its discovery in 1980.

Impairment of mitochondrial acetoacetyl CoA thiolase activity in the colonic mucosa of patients with ulcerative colitis. *Gut*. 2007;56:1543-1549 <https://pubmed.ncbi.nlm.nih.gov/17483192/>

17. Consider including a brief discussion on ethical research reform, perhaps citing the Belmont Report or related ethical guidelines to support your call to reevaluate therapeutic strategies. This is an excellent suggestion.

The recent publications listed below, which are also cited in my manuscript, provide an excellent ethical analysis and offer valuable recommendations for ethical research reform in inflammatory bowel disease. To avoid redundancy, I chose not to reiterate the comprehensive work already conducted by these authors.

In response to the Reviewer's recommendations, however, I included a concise ethical analysis that addresses the prolonged use of non-curative immunosuppressive therapy and its impact on personal autonomy in individuals with chronic ulcerative colitis. To the best of my knowledge, this specific perspective has not been previously explored.

Merza N, Ahmed Z, Nawras M, Dar SH, Itani MI, Al-Hillan A, Zafar Y, Naguib T, Kobeissy AA, Hassan M, Islam A. S946 Ulcerative Colitis Mortality Rate Trends the United States: Two-Decade Analysis Based on US Death Certificates. *ACG*. 2023;118:S709-10.

<https://doi.org/10.14309/01.aig.0000953424.54975.0f>

Din S, Segal J, Blackwell J, Gros B, Black CJ, Ford AC. Harms with placebo in trials of biological therapies and small molecules as induction therapy in inflammatory bowel disease: a systematic review and meta-analysis. *The Lancet Gastroenterology & Hepatology*. 2024;9:1020-1029.

[https://doi.org/10.1016/s2468-1253\(24\)00264-4](https://doi.org/10.1016/s2468-1253(24)00264-4)

<https://pubmed.ncbi.nlm.nih.gov/39307145/>

18. In summary, this extraordinary manuscript brings forth a powerful new model for UC pathogenesis with potentially transformative implications for treatment. It is courageous in its challenge to conventional paradigms and refreshingly humanistic tone. With minor refinements to language, additional scientific substantiation, and clearer figure integration, this work could significantly contribute to a much-needed rethinking in gastroenterology.

The Reviewer is correct. Figure 5 was not appropriately integrated into the text.

I rewrote the last half of the section under the subheading "Loss of redox homeostasis" in the discussion to better integrate the text with figure 5. Figure 6 was also incorrectly labeled as figure 5. I corrected this error.

Please thank the Reviewer for their invaluable suggestions. All are very welcome and will increase the clarity and readability of my paper.

Reviewer # 2, Answer to questions:

I would like to thank the Reviewer for taking the time to review my manuscript. The questions posed regarding very interesting and highly germane to the topic of ulcerative colitis. I have provided answers to all the reviewer's questions below.

1) What is the mechanism of hydrogen peroxide in ulcerative colitis?

Hydrogen peroxide (H_2O_2) is a cell-membrane permeable and a highly potent neutrophilic chemotactic agent that is produced in all cells of the body as a consequence of cellular metabolism. Normally, H_2O_2 is efficiently neutralized by antioxidant enzyme systems in the cell. However, under conditions of oxidative stress exposure, the production of H_2O_2 can overwhelm these antioxidant enzyme systems resulting in H_2O_2 accumulation inside the colonic epithelial cells (colonocytes). When the concentration of H_2O_2 inside the cell is high enough the H_2O_2 can begin to permeate the cell membrane to the colonocyte space and lamina propria.

The H_2O_2 that permeates through the capillaries in the lamina propria is detected by the margined neutrophils on the inner wall of the post capillary venule. Upon contact with H_2O_2 , these neutrophils are stimulated to exit the post capillary venule via diapedesis and, via directed migration, follow the increasing H_2O_2 concentration gradient into the colonic epithelium. Over time, massive numbers of neutrophils enter the colonic epithelium leading to inflammation and ulcerative colitis. This initially occurs in the rectum because the distal colon has the least reductive capacity of the entire large intestine.

2). How does catalase affect the stress response of intestinal epithelial cells?

Catalase can abate the stress response of the cell in at least two ways.

a). The catalase present in peroxisomes can serve as a sink for H_2O_2 generated by enzymes present in the cytoplasm such as cytochrome oxidase, xanthine oxidase and monoamine oxidase.

Cytochrome oxidase P450 enzymes present in the smooth endoplasmic reticulum are active in the metabolism of drugs and xenobiotics, which can generate large amounts of H_2O_2 due to xenobiotic stress. Xanthine oxidase generates H_2O_2 during the metabolism of purines present in red meat and other food stuffs. Finally, monoamine oxidase, present on the outer surface of mitochondria, is active in the metabolism of bioamines such as serotonin, which generates H_2O_2 as byproduct. Large amounts of serotonin are released by enterochromaffin cells present in the colonic epithelium in response to psychological or physical stress.

Thus, catalase can contribute to the metabolism of H_2O_2 generated by xenobiotics, dietary exposures and psychological stress, all of which are oxidative stressors that can initiate or exacerbate ulcerative colitis by increasing the production of H_2O_2 .

b). The high concentration of catalase in peroxisomes is active in neutralizing the H_2O_2 from the metabolism of dietary long-chain acids. A high fat diet is a risk factor for the development or exacerbation of ulcerative colitis. Thus, catalase in peroxisomes is active in reducing the oxidative stress from fat in the diet.

3). What is the specific pathway of hydrogen peroxide in intestinal inflammation?

The origin of H_2O_2 in the cell depends upon the type of oxidative stressor the individual is exposed to. So, for instance, drugs are metabolized via the cytochrome oxidase P450 family of enzymes, which generates H_2O_2 , xenobiotics such as mercury will inactivate thiols such as glutathione leading to an increase in H_2O_2 , pesticides inactivate glutathione peroxidase causing an increase in cellular H_2O_2 stress exposure increases serotonin release by colonic enterochromaffin cells, which generates H_2O_2 via its metabolism by monoamine oxidase after serotonin is internalized by colonocytes, purines in red meat are metabolized by xanthine oxidase with H_2O_2 as a byproduct and smoking cessation causes increased mitochondrial H_2O_2 production due to disinhibition of the electron transport chain resulting in increased electron leakage. Excessive exposure to any of these oxidative stressors may cause increased cellular H_2O_2 buildup and eventual egress from the cell leading to ulcerative colitis as described above.

4). What benefits does the supplementation of catalase bring to patients with ulcerative colitis?

To be effective in ulcerative colitis, catalase would need to be introduced into colonocytes and the colonic lamina propria. Currently, there is no way to do this. Since catalase is a protein, orally administered recombinant catalase would likely undergo enzymatic degradation or inactivation before it reaches the colonocyte. Catalase is a relatively large enzyme, with a molecular weight of around 240 kDa, which makes it unlikely to pass freely through the fenestrations of most capillary beds to reach the colonic lamina propria where H_2O_2 is present in ulcerative colitis. If catalase were to enter circulation from an external source (such as recombinant catalase therapy), it would likely be taken up by cells or cleared before it could move freely across capillary walls. Based on this data, I would not think that exogenous catalase therapy would be helpful for patients with ulcerative colitis.

5). What is the clinical effect of catalase supplements?

I am not aware of catalase supplements being used to treat ulcerative colitis. Catalase supplements are available and marketed for various health benefits, including antioxidant support, anti-aging, and hair health. You can find them in different forms, such as capsules and enzyme drops. Some brands claim that catalase supplements help neutralize hydrogen peroxide, which is linked to oxidative stress and premature graying of hair.

The effectiveness of catalase supplements is still debated. While catalase is a powerful antioxidant enzyme that helps break down hydrogen peroxide in the body, *there is little scientific evidence proving that taking catalase supplements significantly increases enzyme levels or provides substantial health benefits. It appears that the claims are largely based on theoretical benefits rather than strong clinical research. The body naturally produces catalase, and factors like diet and overall health may influence its levels more than supplementation.

6). What are the safety and side effects of catalase supplementation?

Interestingly, intranasal catalase has been administered to mice without toxicity (Hayes) but I have not found any studies using the intravenous route. Oral catalase supplementation is reported to cause GI upset in some individuals. Allergic reaction can occur but are rare. Additionally, the long term side effects of catalase supplementation has not been studied.

Hayes, S.H., Liu, Q., Selvakumaran, S. *et al.* Brain Targeting and Toxicological Assessment of the Extracellular Vesicle-Packaged Antioxidant Catalase-SKL Following Intranasal Administration in Mice. *Neurotox Res* **39**, 1418–1429 (2021).