Dear Editor:

Please express my gratitude to all Peer Reviewers for the time and effort they devoted to reading my manuscript and making recommendations to improve the quality and clarity of the text. I have endeavored to address all issues regarding the preparation of my manuscript for publication. If I have overlooked anything please let me know and I will promptly address it.

Please find my answers to Reviewers below.

Sincerely,

Jay Pravda MD MPH MBE

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade A (Priority publishing)

Conclusion: Minor revision

Specific Comments to Authors: In this paper, the author presents experimental and clinical evidence that identifies hydrogen peroxide produced by the colonic epithelium as the causal agent in the pathogenesis of ulcerative colitis. This paper is well written and the content of the paper is clinically interesting. However, the author should address the following points.

1. With regard to "Evidence based treatment", I suggest that this theme is submitted as an independent paper.

I thank the Reviewer for taking the time to review my manuscript. I agree with the Reviewer that my manuscript is better classified as an independent paper (Review). I shall request that the Editor make this change when re-uploading my revised manuscript.

2. I think that this paper is too long as an article in the academic journal. I suggest that the paper is shorten.

I thank the Reviewer for the feedback regarding the length of my manuscript. Because the pathogenesis is new, researchers will need to view the mechanism from different perspectives in order design experiments that can falsify the mechanism. This is a critical first step towards a universal cure for ulcerative colitis. To this end I have supplied what I feel is a minimal amount of mechanistic and therapeutic detail for researchers to examine and falsify this novel H2O2 based mechanism of disease.

I provide a step-by-step description of the pathogenesis, conceptual redox foundation, and therapeutic rationale along with descriptive diagrams in order to appeal to a multitude of healthcare practitioners all of which will have different levels of experience, training, and educational background. Healthcare practitioners with a lesser degree of preparation will be thankful for the extra effort to explain difficult and new concepts.

Thus, my rationale and justification for maintaining the current manuscript length is because its in-depth explanations will promote inclusiveness of readership to the Journal and facilitate experimental falsification. Both of which are needed to foster understanding of this new mechanism and arrive at a cure.

This manuscript represents the culmination of 17 years of research to understand the mechanism and appropriate treatment of ulcerative colitis. Since publishing my first paper in the World Journal of Gastroenterology in 2005 (1), a great deal of knowledge regarding this new mechanism and the appropriate treatment of ulcerative colitis has been learned. I endeavored to explain this as succinctly as possible while balancing the need to present sufficient explanatory data that is understandable to all levels of healthcare practitioners.

1. Pravda J. Radical induction theory of ulcerative colitis. World journal of gastroenterology: WJG. 2005 Apr 28;11(16):2371. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4305621

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade A (Priority publishing)

Conclusion: Major revision

Specific Comments to Authors: This is an extraordinarily interesting manuscript for the pathogenesis mechanism and treatment of ulcerative colitis. The author put the theory that it is hydrogen peroxide in the colonic epithelial cells but not immune regulation is crucial in the process of ulcerative colitis. This hypothesis does have scientific significance and the author proves the hypothesis in the manuscript well in some aspects. However, this manuscript needs some revision before publication. In this manuscript, the author cited a great number of references. Overall, the abstract can represent the content of this manuscript. The keywords precisely summarized this study. The introduction demonstrated the background of this research, illustrated what is controversial to date, and induced a scientific question. This part supported the content of the manuscript well.

1. A significant limitation of this manuscript is this manuscript was not organized and presented as the structure of evidence-based medicine should be.

Many thanks to the Reviewer for this feedback. I agree, and as indicated below, I have changed the classification of my manuscript to a review, which as the Reviewer correctly points out is a more appropriate classification for my manuscript.

2. For the title, the scientific hypothesis of this article is the enrichment of hydrogen peroxide in the colonic epithelial cells drives inflammatory bowel disease and the intervention of pathological hydrogen peroxide signaling can lead to long-term relief of ulcerative colitis. I think the title does not reflect this hypothesis comprehensively and thoroughly.

The Reviewer is correct and I thank the Reviewer for this observation. I have modified the title to make it more reflective of the content of the manuscript.

3. In the abstract, the conclusion of "Cumulative data indicate that individuals with ulcerative colitis have normal immune systems and current treatment guidelines calling

for modification of the immune response based on the belief that ulcerative colitis is caused by an underlying immune dysfunction is not supported by the evidence and potentially very harmful" might be too radical; because some studies have indicated the immune factors played a role in the pathogenesis process of ulcerative colitis, and there might not sufficient evidence of the harmfulness of immune therapy to ulcerative colitis patients.

The Reviewer makes a good point. I have limited my description to agents that suppress the immune response which cause the most serious adverse effects. I have modified my description to state that immunosuppressive agents may cause serious adverse effects, which I believe most healthcare providers would agree with.

Some examples are agents such as Janus Kinase inhibitors (i.e. Tofacitinib (Xeljanze) to which the FDA added a black box warning in 2021 because of the increased risk of heart attack, stroke, cancer, blood clots and even death. Other immunosuppressive agents can cause bone marrow suppression. Biologics can cause lymphoma or serious viral infections such as progressive multifocal leukoencephalopathy. Thus, some mention of the serious side effects of these agents is warranted. I thank the Reviewer for pointing this out.

4. Other problems are listed below. 1. As is mentioned above, the organization of this manuscript is more likely a review instead of an evidence-based medicine study.

I thank the Reviewer for this suggestion. I agree. I have asked the editor to reclassify my manuscript as a review.

5. In each part the author explained the background, including some possible hypotheses, and proved it via references, making it probably a structure of a literature review. The author does cite numerous references, but there might be a lack of literature screening and evidence level determination process.

I agree with the Reviewer that my manuscript should be reclassified as a review and I have asked the Editor to do so. Regarding literature screening, I cite at least three studies with hard experimental evidence strongly implicating H2O2 as the causal agent in ulcerative colitis.

In the first study (1) the authors conclude:

"A "radical induction" theory of ulcerative colitis has recently been proposed, which hypothesises 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 also resolved a decades-old mystery regarding the impairment of beta oxidation in the colonic epithelium of people with ulcerative colitis. The study verified my prediction that H2O2 induced oxidative inhibition of mitochondrial thiolase is responsible for impaired beta oxidation in individuals with ulcerative colitis. This prediction appeared in my first paper in which I first proposed an H2O2 based pathogenesis of ulcerative colitis that I named "Radical Induction theory". This paper was published in the World Journal of Gastroenterology 17 years ago in 2005 (2).

In the second study the authors arrive at the following conclusion regarding a causal role for H2O2 in the pathogenesis of ulcerative colitis (3):

"On the basis of extensive scientific and clinical evidence accumulated over decades, Pravda makes a compelling case that increase in the production of oxidative stress in epithelial cells followed by diffusion to its microenvironment, damage to tight junctions and local accumulation of white blood cells underlie the etiology of ulcerative colitis (radical induction theory of ulcerative colitis). Our findings support and extend this theory…"

These two independent research groups provide hard experimental evidence that strongly implicates a causal role for H2O2 in the pathogenesis of ulcerative colitis. Additionally, glutathione peroxidase knock-out mice (that cannot eliminate H2O2) develop an ulcerative colitis type of colonic inflammation. I cite all three studies in my manuscript.

The third study is the experimental demonstration of colitis in glutathione peroxidase knock-out mice (4). The mice cannot eliminate H2O2, which accumulates in the colon causing colonic inflammation similar to human ulcerative colitis. I also cite additional evidence in my manuscript.

In contrast, despite over 50 years of intense research, no immune abnormality has ever been found to support immune dysregulation as the cause of ulcerative colitis so an evidence-based search would not be fruitful. Instead I performed a comparative review based on explanatory power and predicted therapeutic development for each of the two proposed mechanisms, which is more appropriate and productive.

1. Impairment of mitochondrial acetoacetyl CoA thiolase activity in the colonic mucosa of patients with ulcerative colitis. https://pubmed.ncbi.nlm.nih.gov/17483192/

2. Pravda J. Radical induction theory of ulcerative colitis. World journal of gastroenterology: WJG. 2005 Apr 28;11(16):2371. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4305621/

3. Differential immune and genetic responses in rat models of Crohn's colitis and ulcerative colitis
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3025515/

4. Esworthy RS, Aranda R, Martin MG, Doroshow JH, Binder SW, Chu FF. Mice with combined disruption of Gpx1 and Gpx2 genes have colitis. *Am J Physiol Gastrointest Liver Physiol.* 2001; **281:** G848-855.
https://pubmed.ncbi.nlm.nih.gov/11518697/

6. If the author wants to publish this manuscript as an evidence-based medicine study but without PRISMA 2009 Checklist, it might be imperative to explain the reason.

I have reclassified my manuscript as a review, which obviates the need for this additional documentation. I am grateful to the Reviewer for this observation.

7. Some of the subtitles are intriguing but cannot summarize the subsequent content, such as 1.1 and 4.6.

I agree with the Reviewer that the subtitles may not convey the same message to all readers. I greatly appreciate this recommendation. I have modified the subtitles to reflect the underlying content of the section.

8. Part 5 mentioned "evidence-based" treatment, but in the result part (5.3), only one patient was reported. Basically, it might be imperative to provide the demographic data and the inclusion/exclusion criteria of these patients. If the author wants to convince the readers the study design is indeed evidence-based, I think the author should add the method part to illuminate the study design. And some statistical description might be also needed.

We did not conduct a study. Patients with refractory ulcerative colitis were treated as part of the practice of medicine. I thank the Reviewer for pointing out this confusion and I have clarified the wording in the manuscript to reflect this distinction.

As an aside, these patients had run out of medical options and were facing the real prospect of a total colectomy before receiving the therapy. Section 5.3 of the manuscript describes our experience regarding 36 patients with refractory ulcerative colitis, 85% of which achieved complete histologic remission within weeks. Section 5.3 also contains a case report of a single patient with a 39 year history of refractory ulcerative colitis and continuous intermittent rectal bleeding who was weeks away from a total colectomy. The patient provided us with an unsolicited colonoscopy report 12 years after receiving the treatment. The colonoscopy and biopsy were completely normal.

This long duration of complete histologic remission is extremely unlikely to have occurred by chance and strongly suggests that therapy to restore colonic epithelial redox homeostasis by normalizing intracellular colonocyte H_2O_2 can be curative in patients with ulcerative colitis. A H_2O_2 based pathogenesis of ulcerative colitis predicts that a cure is possible and identifies the molecular mechanism that can achieve this outcome, which I explain in my manuscript.

9. In conclusion, this manuscript might be more likely publishing as a review instead of an evidence-based study under the author's current work and some revisions.

I agree and I thank the Reviewer for this valuable feedback. I have asked the editor to change the classification of my manuscript to a review.

EDITORIAL OFFICE'S COMMENTS:

Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:

Science editor:

1. The manuscript is more like a review than an evidence-based medical study.

Thank you for this feedback. I have changed the classification of my manuscript to a review, which is more appropriate.

2. Although the author cited a large number of references, he lacked the essence of the cited documents and reorganized useful information according to the needs of his article.

I assume the Reviewer uses the word "essence" to mean extracting the most pertinent data from each reference to support the claims made in the manuscript.

In fact, this is what I did. I read every one of the more than 300 references that I cited. I extracted the pertinent data from each reference in support of the evidence-based novel pathogenesis of ulcerative colitis that I present in my manuscript. With this large number of references, one must be highly focused on the appropriate and relevant information to prevent unintentional expansion of the scope that originally defined the manuscript. I endeavored to do that by limiting my thesis to the central themes of pathogenesis and therapeutics with enough accompanying conceptual explication for readers to understand these new concepts that form the foundation of redox medicine. Organizing the presentation of this new pathogenesis presented some challenges because the oxidative stressor(s) exposure that initiates the process leading to ulcerative colitis is unrecognized and occurs some time before the illness is diagnosed. Additionally, although relapse manifests month to years after diagnosis, its cause was

set in motion much earlier around the time of diagnosis. Thus, I elected to present the pathogenesis underlying these events starting with the histological diagnosis (which readers are familiar with) and develop the pathogenesis from that point. However, I also reprise the entire pathogenesis in sequential stepwise fashion in figure # 3 so readers can visualize the entire natural history of this condition from its inception.

3. No PRISMA 2009 Checklist.

As per the Reviewer's recommendation, I have reclassified my manuscript as a review so it is neither a systemic review nor a meta-analysis, and thus the PRISMA checklist would not apply.

4. If this manuscript is too long for an article, I suggest it be simplified.

I understand the Reviewer's concerns. However, I am presenting a new paradigm that has the potential of converting a here to forth "incurable" disease (ulcerative colitis) into an easily treatable and potentially curable condition. This type of endeavor requires some expository development in order to answer the many questions that will inevitably arise in readers' minds. Given the nature of the manuscript, a degree of flexibility regarding the length is needed to adequately present the data and concepts. Additionally, not every reader will have the same degree of medical and basic science background to adequately understand the concepts that form the basis of this novel pathogenesis of ulcerative colitis. I endeavored to include sufficient information to make the manuscript as inclusive as possible for all healthcare providers involved in the care and treatment of ulcerative colitis. I want readers of all levels of expertise to understand the mechanism and treatment rationale. Thus, I honestly feel that the length of the manuscript is justified by the inclusiveness of the additional readership that it will bring to the Journal.

5. Language Quality: Grade C (A great deal of language polishing)

I thank the Reviewer for this observation. I have proofread my manuscript again and have made improvements to the syntax and punctuation to better convey the important concepts presents in the text of the paper. I have taken the additional step of improving my text with professional grammar correcting software to incorporate additional clarity.

Company editor-in-chief:

I have reviewed the Peer-Review Report, full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Gastroenterology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors.

Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file. **Done.**

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