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In response to comments and suggestions of Reviewer A

Reviewer A:

Despite summarizing major roles for Magnesium isoglycyrrhizinate in controlling inflammation and reducing end-organ damage, there are no evidence of the potential role for this drug as adjuvent to any convential treatment for viral infections affecting the respiratory system. In light of the absence of such substantial elements, one cannot advocate for a role of such a drug in any treatment mangment for COVID-19.
This review would have been of general value but oustide the COVID-19 context.

Reply 1: In this review, we summarize beneficial effects of Magnesium isoglycyrrhizinate clinical application and basic experimental research, including antiviral effect, inhibition of cytokine storm and and oxidative stress, improvement of multiple organ damage. And based on these data, we speculate that magnesium isoglycyrrhizinate may have an auxiliary role in the treatment of COVID-19.
In terms of antiviral effect, glycyrrhizic acid compounds have been confirmed to have antiviral and immunomodulatory effects, mainly through inhibiting the process of viral replication, adhesion and infection. Of note, Glycyrrhizic acid compounds have been used to treating COVID-19 patients, with antibody test from positive to negative. Glycyrrhizic acid metabolizes into glycyrrhetinic acid, which also is the metabolite of magnesium isoglycyrrhizinate. When SARS-CoV-2 infects host cells, glycyrrhetinic acid can inhibit the expression of TMPRSS2, thereby suppressing the priming effect of surface S protein. Mover, magnesium isoglycyrrhizinate could reduce viral load, inhibit virus proliferation and enhance immunity to accelerate the recovery of liver function for the treatment of viral hepatitis in clinic. Importantly, magnesium isoglycyrrhizinate is now included as part of guidelines issued by the National Health Commission of the People's Republic of China. However, there is still no report on the detailed clinical

data of magnesium isoglycyrrhizinate as SARS-COV2 antiviral drug. This is completely understandable. At present, there is little research on the extensive antiviral mechanism of magnesium isoglycyrrhizinate, mainly focusing on viral hepatitis. We speculate the possible antiviral effect and mechanism of magnesium isoglycyrrhizinate on SARS-CoV2 based on glycyrrhizic acid, just like other drugs that may have therapeutic effects on COVID-19 in the published literatures.

Indeed, we cannot give decisive conclusions that magnesium isoglycyrrhizinate could inhibit SARS-CoV infection. We have revised the narrative about antiviral effects to express our meaning more clearly.

Changes in the text:

This narrative review provides the evidence supporting the recommended MgIG as supportive therapy in the "Management Standard for Mild and Common Patients of Coronavirus Disease 2019 (COVID-19) (Second Edition)", which is jointly issued by National Health Commission of People's Republic of China and National Administration of Traditional Chinese Medicine (see Page 29, line 610-615).

Moreover, we have modified the question mark in Figure 2 and Figure 4 on the efficacy of MgIG in inhibiting viral replication, making the description of this article more accurate (see Figure 2 and Figure 4).

And we further updated COVID-19 data (see Page 2, line 24 and Page 29, 604).

In response to comments and suggestions of Reviewer B

Reviewer B:

This narrative review highlights a compound which has been used for other disease processes and is now included as part of guidelines issued by the National Health Commission of the People's Republic of China. The review details some of the beneficial effects that the compound, MgIG can have on patients with various conditions, and how those beneficial effects could apply to COVID-19 patients. The primary purpose of the review is to summarize the supporting evidence behind the recommendation of the use of MgIG, and perhaps to recommend further study into the use of this compound in patients with COVID-19.

Overall, the presented information provides a good framework for MgIG and cites some

of the mechanisms that MgIG is proposed to provide benefit to various organs in distress. The provided figures are helpful in understanding some of the mechanisms, however, I would also like a graphical representation of the available clinical data if that is possible.

There are numerous grammatical errors throughout the manuscript, which could be ironed out with a thorough copy-editing. Repair of said errors will greatly enhance the clarity of the manuscript.

Given the presented manuscript, I would recommend a major revision regarding extensive grammatical corrections, as well as a helpful figure detailing some of the data/clinical trials that MgIG has previously been used in.

Reply: First, Thanks for your affirmation of our work. And thanks for your careful and constructive opinions. Below we will make detailed revisions to your suggestions and comments.

1. Minor grammatical corrections throughout the manuscript recommended, for example, in the abstract, “in a large amount and reach high-titer levels” should read “in a large amount and reaches high-titer levels”.

Reply 1: Thanks.

Changes in the text: We have modified our text as advised (see Page 4, line 56).

2. In abstract, “cytokine storm and multi-organ damage even death” may read “cytokine storm, multi-organ failure, and even death.”

Reply 2: Thanks.

Changes in the text: We have revised it according to your suggestion (see Page 2, line 30).

3. In abstract, “no effective measure” should read “no effective measures”.

Reply 3: Thanks.

Changes in the text: We have revised it (see Page 2, line 31).

4. Please ensure adequate spacing, on line 61 “, and lead to extrapulmonary manifestations” may read as “and can lead to extrapulmonary manifestations”.

Reply 4: Thanks for your careful review.

Changes in the text: We have added adequate spacing and revised inappropriate language expression as your suggestion. Patients with moderate to severe COVID-19 show a cytokine storm, which causes pathological changes of the respiratory system, and leads to extrapulmonary manifestations (see Page 5, line 69).

5. Subheading 1 (line 89) could have a more concise title, such as "Protecting Vital Organs".

Reply 5: Thanks for your careful review.

Changes in the text: We have modified our text as advised (see Page 6, line 101).

6. Subheading 1.1 (line 90), "liver" should be capitalized.

Reply 6: Thanks.

Changes in the text: We have modified our text as advised (see Page 6, line 102).

7. Under subheading 1.1, please propose a mechanism for how MgIG helps/enhances antihepatitis activity. Perhaps include some of the data from the studies cited.

Reply 7: We have added some content to propose a mechanism for how MgIG helps/enhances antihepatitis activity.

Changes in the text: MgIG effectively prevents and treats liver diseases such as viral hepatitis, drug hepatitis, alcoholic hepatitis, and liver fibrosis possibly through inhibiting transcription factor kappa B (NF-κB), IL-6, tyrosine protein kinase 2/signal transducer and transcription activator 3 (JAK2/STAT3) signaling pathways. (see Page 6, line 106-108).

8. For drug agents not previously mentioned, please provide a brief definition statement, such as for Tiopronin on line 112.

Reply 8: Thanks. We have provided a brief definition statement of Tiopronin and Lamivudine.

Changes in the text: Tiopronin is used in the early treatment of viral hepatitis, alcoholic hepatitis, drug-induced hepatitis, heavy metal toxic hepatitis, severe hepatitis and liver cirrhosis, as standard therapy for liver injury in China (see Page 8, line 136-138).

Lamivudine is often used for the treatment of adult chronic hepatitis B patients with compensatory liver function accompanied by elevated alanine aminotransferase and viral activity replication (see Page 7, line 123-125).

9. Under subheading 1.1.1 please propose a mechanism for the hepatoprotective effects of MgIG in the setting of hepatitis.

Reply 9: Thanks for your reminder.

Changes in the text: Of note, studies of MgIG on viral hepatitis are mostly clinical trials, but the corresponding basic experiments are scarce. The mechanism for hepatoprotective effects of MgIG in the setting of hepatitis is worthy of further exploration (see Page 7, line 127-130).

10. Noted a mechanism proposed for drug induced hepatitis in 1.1.2 regarding oxaliplatin-induced liver injury as well as doxorubicin-induced hepatotoxicity; if possible, please include some form of proposed mechanism for other hepatoprotective effects of MgIG versus other drugs.

Reply 10: Thanks for your nice suggestion.

Changes in the text: We have modified our text and added corresponding mechanisms as advised. MgIG (15 or 45 mg/kg ip, qd) also prevents oxaliplatin-induced liver injury. It inhibits mRNA and protein expression of liver metallothionein 1, peroxiredoxin 1, superoxide dismutase 2, IL-6 and STAT3, and suppresses mRNA and protein expression of liver von Willebrand factor, plasminogen activator inhibitor-1 in this animal model, thus showing its attention of liver injury-related oxidative stress, IL-6 pathway activation and coagulation system disturbances [1]. (see Page 8, line 149-154). MgIG also ameliorate doxorubicin-induced acute hepatotoxicity via anti-oxidant and anti-apoptotic mechanisms in mice [2]. MgIG significantly increases SOD and GSH Px and decreases MDA levels in serum, and down-regulates liver expression levels of apoptosis-related proteins Bax/Bcl-2, and caspase-3 in doxorubicin-treated mice [2] (see Page 9, line 162-164).

[1]. Zou X, Wang Y, Peng C, et al. Magnesium isoglycyrrhizinate has hepatoprotective effects in an oxaliplatin-induced model of liver injury. Int J Mol Med. 2018;42(4):2020-2030.

[2]. Wu, Z., Zhang, Y., Song, T., Song, Q., Zhang, Y., Zhang, X., Han, X., Zhang, J., &

Chu, L. (2018). Magnesium isoglycyrrhizinate ameliorates doxorubicin-induced acute cardiac and hepatic toxicity via anti-oxidant and anti-apoptotic mechanisms in mice. Experimental and therapeutic medicine, 15(1), 1005–1012.

11. On line 137, would suggest changing “can significantly treats alcoholic hepatitis” to “can help treat alcoholic hepatitis..

Reply 11: Thanks.

Changes in the text: Thanks. We have modified our text as advised (see Page 9, line 174).

12. On line 139, “showing its better therapeutic effect” could read “showing an enhanced therapeutic effect”.

Reply 12: Thanks.

Changes in the text: We have modified our text as advised (see Page 9, line 176).

13. Noted a mechanism proposed for alcoholic liver injury protection involving inhibited transduction of the hedgehog signaling pathway, does this explain the entirety or is there more to alcoholic liver injury protection?

Reply 13: Thanks for your question. There are three hedgehog homologous genes in mammals: Sonic Hedgehog (Shh), Indian Hedgehog (Ihh) and Desert Hedgehog (Dhh), which encode Shh, Ihh and Dhh proteins, respectively. hedgehog signaling is controlled by two receptors on the target cell membrane, Patched (Ptc) and Smoothened (Smo), and in turn regulating downstream genes like Ptch1, CCND1, CCND2, FOXM1, Bcl2, FOX2. As mentioned in the manuscript, hedgehog signaling is related to steatosis, oxidative stress, mitochondrial dysfunction, and apoptosis in hepatocytes in ethanol-induced liver injury model [1]. However, studies on the protection of MgIG against alcohol-induced liver damage is still lacking. We cannot conclude that the protection of MgIG from alcoholic liver injury is all due to regulation of the hedgehog pathway.

[3]. Lu C, Xu W, Shao J, et al. Blockade of hedgehog pathway is required for the protective effects of magnesium isoglycyrrhizinate against ethanol-induced hepatocyte steatosis and apoptosis. IUBMB Life. 2017;69:540-52.

Changes in the text: However, However, more studies are needed to explore possible

molecular mechanisms by which MgIG protects from alcoholic hepatitis (see Page 10, line 186).

14. Noted the proposed mechanisms in section 1.1.4, would suggest grammatical editing, otherwise helpful.

Reply 14: Thanks.

Changes in the text: We have modified our text as advised. In animal model of carbon tetrachloride (CCl4)-induced liver fibrosis, MgIG (15, 30 or 45 mg/kg ip, qd) markedly prevents hepatic injury and reduces fibrotic scar formation, further results from in vitro experiments show that the anti-fibrosis effect of MgIG (2.5, 5, 10 mg/ml) may be associated with its regulation of hepatic stellate cell (HSC) ferroptosis via a HO-1 dependent mechanism (see Page 10, line 191-193).

15. Line 191-192 the phrase “Cardiac hypertrophy as the independent risk factor of increasing cardiovascular disease mortality threatens human” could read “Cardiac hypertrophy is an independent risk factor of increasing cardiovascular disease mortality and threatens human”.

Reply 15: Thanks.

Changes in the text: We have modified our text as advised (see Page 12, line 238).

16. Line 202, “Kidney is an important organ” could read, “The kidney is an important organ”.

Reply 16: Thanks.

Changes in the text: We have modified our text as advised (see Page 13, line 250).

17. Line 205, “MgIG has good intervention effects” could read “MgIG” has positive effects on patients with”.

Reply 17: Thanks.

Changes in the text: We have modified our text as advised (see Page 13, line 253).

18. Later lines discuss lesions related to pulmonary fibrosis in subheading 1.4, but please clarify in the first sentence the meaning of “lung lesions”. Unclear as to

meaning of "reported early".

Reply 18: Sorry about this problem. The current research on the protective effect of MgIG on the lung is mainly focused on pulmonary fibrosis. Pulmonary fibrosis is also a kind of lung lesion. Our language description is not accurate enough and has been revised.

Changes in the text: We have modified the description of the first sentence. The protective effect of MgIG on paraquat and radiation-induced pulmonary fibrosis is reported [4,5](see Page 14, line 268).

[4]. Xiao ZW, Zhang W, Ma L, Qiu ZW. Therapeutic effect of magnesium isoglycyrrhizinate in rats on lung injury induced by paraquat poisoning. Eur Rev Med Pharmacol Sci. 2014;18(3):311-320.

[5]. Yang Q, Zhang P, Liu T, et al. Magnesium isoglycyrrhizinate ameliorates radiation-induced pulmonary fibrosis by inhibiting fibroblast differentiation via the p38MAPK/Akt/Nox4 pathway. Biomed Pharmacother. 2019;115:108955.

19. Line 247, "there is no specialized" should read "there are no specialized".

Reply 19: Thanks.

Changes in the text: We have modified our text as advised (see Page 15, line 300).

20. Line 248, please keep a standardized way of spelling "COVID-19".

Reply 20: Thanks.

Changes in the text: We have modified our text as advised (see Page 15, line 301).

21. Line 251-252, please mention a citation such as guideline or news report.

Reply 21: Thanks.

Changes in the text: We have added corresponding citations (see Page , line). The medications used for COVID-19 include remdesivir, chloroquine and hydroxychloroquine, tocilizumab, lopinavir/ritonavir, favipiravir, convalescent plasma therapy, azithromycin, vitamin C, corticosteroids, interferon and colchicine (6-14) (see Page 16, line 306).

[6]. Ramiro S, Mostard RLM, Magro-Checa C, et al. Historically controlled

comparison of glucocorticoids with or without tocilizumab versus supportive care only in patients with COVID-19-associated cytokine storm syndrome: results of the CHIC study. Ann Rheum Dis. 2020;79:1143-1151.

[7]. Hung IF, Lung KC, Tso EY, et al. Triple combination of interferon beta-1b, lopinavir-ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised, phase 2 trial. Lancet. 2020;395:1695-1704.

[8]. Irie K, Nakagawa A, Fujita H, et al. Pharmacokinetics of favipiravir in critically Ill patients with COVID-19. Clin Transl Sci. 2020;13:880-885.

[9]. Deftereos SG, Giannopoulos G, Vrachatis DA,et al. Effect of colchicine vs standard care on cardiac and inflammatory biomarkers and clinical outcomes in patients hospitalized with coronavirus disease 2019: the GRECCO-19 randomized clinical trial. JAMA Netw Open. 2020;3:e2013136.

[10]. Damle B, Vourvahis M, Wang E, et al. Clinical pharmacology perspectives on the antiviral activity of azithromycin and use in COVID-19. Clin Pharmacol Ther. 2020;108:201-211.

[11]. Liu F, Zhu Y, Zhang J, Li Y, Peng Z. Intravenous high-dose vitamin C for the treatment of severe COVID-19: study protocol for a multicentre randomised controlled trial. BMJ Open. 2020;10:e039519

[12]. Wang Y, Zhang D, Du G, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet.

2020;395:1569-78.

[13]. Boulware DR, Pullen MF, Bangdiwala AS, et al. A randomized trial of hydroxychloroquine as postexposure prophylaxis for COVID-19. N Engl J Med. 2020;383:517-25.

[14]. Li L, Zhang W, Hu Y, et al. Effect of convalescent plasma therapy on time to clinical improvement in patients with severe and life-threatening COVID-19: a randomized clinical trial. Jama. 2020;324:1-11.

22. Line 269, “assesse” should be “assessed”.

Reply 22: Thanks.

Changes in the text: We have modified our text as advised (see Page 16, line 325).

23. Line 275, “In a randomized trial enrolled” should read “In a randomized trial which enrolled”. Additionally, on line 276 “hydroxychloroquine or placebo is” should read “hydroxychloroquine or placebo were”.

Reply 23: Thanks.

Changes in the text: We have modified our text as advised (see Page 17, line 333-335).

24. Line 308, “h has” appears to be a fragment, would remove the “h”.

Reply 24: Thanks.

Changes in the text: We have modified our text as advised (see Page 18, line 372).

25. Line 319 and 320, please clarify what is meant by “past 7 months” and “two previous coronavirus” in this context.

Reply 25: Thanks.

Changes in the text: We have deleted "within the past 7 months" and added the meaning of “two previous coronavirus”. The two previous coronavirus refer to SARS-CoV and MERS-CoV (see Page 19, line 386-387).

26. Line 333, “Gglycyrrhizin” appears to be an autocorrection error.

Reply 26: Thanks.

Changes in the text: We have modified our text as advised (see Page 20, line 401).

27. Line 340, same as above.

Reply 27: Thanks.

Changes in the text: We have modified our text as advised (see Page 20, line 404).

28. Example provided in lines 342-344 is thematically out of place. Would place this toward the end of the discussion on diammonium glycyrrhizinate activity or change phrasing of “of note” to match with a brief introductory statement.

Reply 28: Thanks for your comments.

Changes in the text: We have changed phrasing of “of note” to match with a brief introductory statement, and we place this description toward the end of the discussion at the end of this paragraph.

MgIG is often used in the treatment of viral hepatitis. It inhibits the replication of hepatitis virus, showing its better effect than previous generations of glycyrrhizic acid preparations (see Page 21, line 421).

29. Line 355, is there already a relation to ACE2 and MgIG?

Reply 29: No relevant research has appeared yet. The relationship between MgIG and ACE2 is worth exploring.

Changes in the text: Are these effects related to the inhibition of ACE2? It is worth exploring (see Page 21, line 426).

30. Line 357, “also known as cytokine cascade” should be separated by commas. Does not need a comma at “response, which”.

Reply 30: Thanks.

Changes in the text: We have modified our text as advised (see Page 21, line 429).

31. Line 360, “multiple organs failure” should be “multiple organ failure.”

Reply 31: Thanks.

Changes in the text: We have modified our text as advised (see Page 21, line 431).

32. Consider naming the clinical trials in the text where MgIG has been directly used.

Reply 32: Thanks. The Phase III clinical trial of MgIG injection for the treatment of viral hepatitis dominated by Chia Tai Tianqing Pharmaceutical Group, did not mention the name of the clinical trial at the time of application [15]. And the phase I clinical trial evaluates the pharmacokinetics and safety of MgIG after single and multiple intravenous doses by infusion in healthy volunteers, but the article also did not mention in detail the name of the clinical trial at the time of application [16].

Changes in the text: We have added the company responsible for the clinical trial. The corresponding content has been mentioned or modified in the text (see Page 27, line 572).

[15]. Chia Tai TianQing. The Instructions of Magnesium Isoglycyrrhizinate Injection [Internet]. Chia Tai Tian Qing Pharmaceutical Co., Ltd. 2014, cited 2020 Oct 24. Available from: https://www.cttq.com/product/baogan-detail-6009.htm

[16]. Sun L, Shen J, Pang X, Lu L, Mao Y, Zeng M. Phase I safety and pharmacokinetic study of magnesium isoglycyrrhizinate after single and multiple intravenous doses in chinese healthy volunteers. J Clin Pharmacol. 2007;47(6):767-773.

33. Line 413, should be “SARS-CoV-2” rather than “SARA-CoV-2”.

Reply 33: Thanks. Sorry for this typographical error.

Changes in the text: We have modified our text as advised (see Page 24, line 492).

34. For Line 446-453, if possible please cite information available on the clinical significance of flocs produced from mixing solutions.

Reply 34: Combination of drugs is common in clinical practice, but drug interactions are prone to occur. The flocculation produced by the combination of MgIG injection and other drugs for COVID-19 patients indicates that the compatibility between these drugs is poor. There may be a chemical reaction, which is prone to adverse reaction. In addition, these concentrations of drug combination will also be affected. When this happens, it is recommended to stop the medication (MgIG injection combined with

other drugs) and adopt a separate interval administration method for COVID-19 patients (see Page 26, line 541-548).

Changes in the text: We have added corresponding descriptions and explanations in the test as advised (see Page 25, line 541-548).

35. Line 475, please mention the name or primary author of the viral hepatitis trial involved with MgIG.

Reply 35: The phase III clinical trial of 332 cases of viral hepatitis was dominated by Chia Tai Tianqing Pharmaceutical Group Co., Ltd., and we have included the main information in the manuscript [15].

Changes in the text: Corresponding details have been revised in the manuscript (see Page 27, line 572).

[15]. Chia Tai TianQing. The Instructions of Magnesium Isoglycyrrhizinate Injection [Internet]. Chia Tai Tian Qing Pharmaceutical Co., Ltd. 2014, cited 2020 Oct 24. Available from: https://www.cttq.com/product/baogan-detail-6009.htm

36. For discussion, please mention limitations from the data of different clinical trials discussed.

Reply 36: Thanks. Indeed, we lacked discussion of the limitations of clinical trials, so we added these contents of different clinical trials.

Changes in the text: Clinical trials have the limitations. Phase I clinical trial shows the safety of MgIG injection, but the supporting populations are all from China. For people from other countries, further experiments and clinical evidence are needed. And as mentioned above, the Phase III clinical trial of MgIG led by Chia Tai Tianqing is mainly for patients with viral hepatitis, and lacks universality for other types of hepatitis [16]. We have modified our text as advised (see Page 28, line 581-586).

[16]. Sun L, Shen J, Pang X, Lu L, Mao Y, Zeng M. Phase I safety and pharmacokinetic study of magnesium isoglycyrrhizinate after single and multiple intravenous doses in chinese healthy volunteers. J Clin Pharmacol. 2007;47(6):767-773.

37. Appreciate the information provided in Figure 2. If possible, please provide citations.

Reply 37: Thanks.

Changes in the text: We have added in Figure 2 as advised (see Figure 2).

38. Appreciate the information provided in Figure 4. If possible, please provide citations.

Reply 38: Thanks. Since Figure 4 is a summary picture of the full text, it contains almost all literature references, so we have not added references here. Thank you for your suggestion. But we have added the corresponding references in Figure 2 and Figure 3.

39. Appreciate the information provided in Table 2. If possible, please provide citations

Reply 39: Thanks for your comments. We have added the references in Figure 2 and Figure 3. The table 2 summarize the effects of MgIG on tissue and organ in the text, and the references are consistent with the Part 1, “protecting vital organs”, so the references are not added in Table 2. And we have added a note below table 2.

Changes in the text: We have added in Figure 2 and Figure 3 as advised (see Figure 2 and Figure 3). And we have added a note below table 2 (see in Table 2).