European Review for Medical and Pharmacological Sciences 2020; 24: 10228-10238

Could dantrolene be explored as a repurposed drug to treat COVID-19 patients by restoring intracellular calcium homeostasis?

B. JIANG[1,2], S. LIANG[1,3], G. LIANG[1], H. WEI[1]

[1]Department of Anaesthesiology and Critical Care, University of Pennsylvania, Philadelphia, PA, USA
[2]Department of Anaesthesiology, Peking University People's Hospital, Beijing, China
[3]Department of Anesthesiology, the First Affiliated Hospital of Jinan University, Guangzhou, China

Bailin Jiang and Shuqing Liang contributed equally to this paper

Abstract. – Dantrolene, an FDA approved drug to treat malignant hyperthermia and muscle spasm, has been demonstrated to inhibit severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mediated toxicity of host cells. Ryanodine receptor overactivation and associated disruption of intracellular Ca^{2+} homeostasis play important roles in SARS-CoV-2 infection and replication of host cells. Dantrolene, as an inhibitor of RyRs, is expected to ameliorate these detrimental effects of SARS-CoV-2 in host cells. Additionally, dantrolene has also been shown to inhibit multiple cell or organ damage induced by hypoxia/ischemia, mitochondria damage, oxidative stresses, inflammation, impairment of autophagy and apoptosis, etc., which are often the causes of severity and mortality of COVID-19 patients. We have repurposed that dantrolene has a high potential at treating COVID-19 patients and reducing its morbidity and mortality.

Key Words:
SARS-CoV-2, COVID-19, Infection, Replication, Dantrolene.

Introduction

The epidemic of coronavirus disease 2019 (COVID-19) caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has lasted more than half a year with varied population-level case fatality ratio ranged between 2-8%[1], though much lower after adjusting for demography and under-ascertainment, which is still higher in aged groups (≥60 years: 6·4%, ≥80 years: 13·4%)[1]. Nevertheless, there is still a lack of powerful drugs to treat COVID-19 patients, even with the promising drug Remdesivir, a nucleotide analog with broad-spectrum antiviral activity[2]. Furthermore, randomized clinical trials have also shown disappointing findings of other drugs, including hydroxychloroquine[3] and lopinavir-ritonavir[4]. In the setting of the absence of robust drug and vaccine, it may be beneficial to develop drugs that can reduce the infection and replication of SARS-CoV-2 and severity of the symptoms[5], protect the organs, ameliorate the deterioration[6] and reduce mortality in the critically ill COVID-19 patients[7]. Considering its plausible ability to inhibit SARS-CoV-2 virus cytotoxicity of host cells[8], cytoprotection[9], and organ protection[10] in a wide variety of models of stress and disease, we propose that dantrolene, an FDA approved drug to treat malignant hyperthermia and muscle spasm, could be repurposed as a potential adjuvant drug for the treatment of COVID-19 patients.

1. Potential and Proposed Mechanisms of dantrolene to inhibit SARS-CoV-2 Infection and/or Replication in the Host Cells

Infection and replication of SARS-CoV-2 (Figure 1) in the host cells initially require binding of the S1 domain of the virus spike protein (S protein) to angiotensin-converting enzyme 2 (ACE2) on the plasma membrane, followed by fusion with the plasma membrane mediated by S2 domain of S protein to make its entry[11,12].

The cleavage and activation of S protein by protease, especially cathepsin L[13], provides a pre-

Corresponding Author: Huafeng Wei, MD, Ph.D; e-mail: Huafeng.wei@pennmedicine.upenn.edu

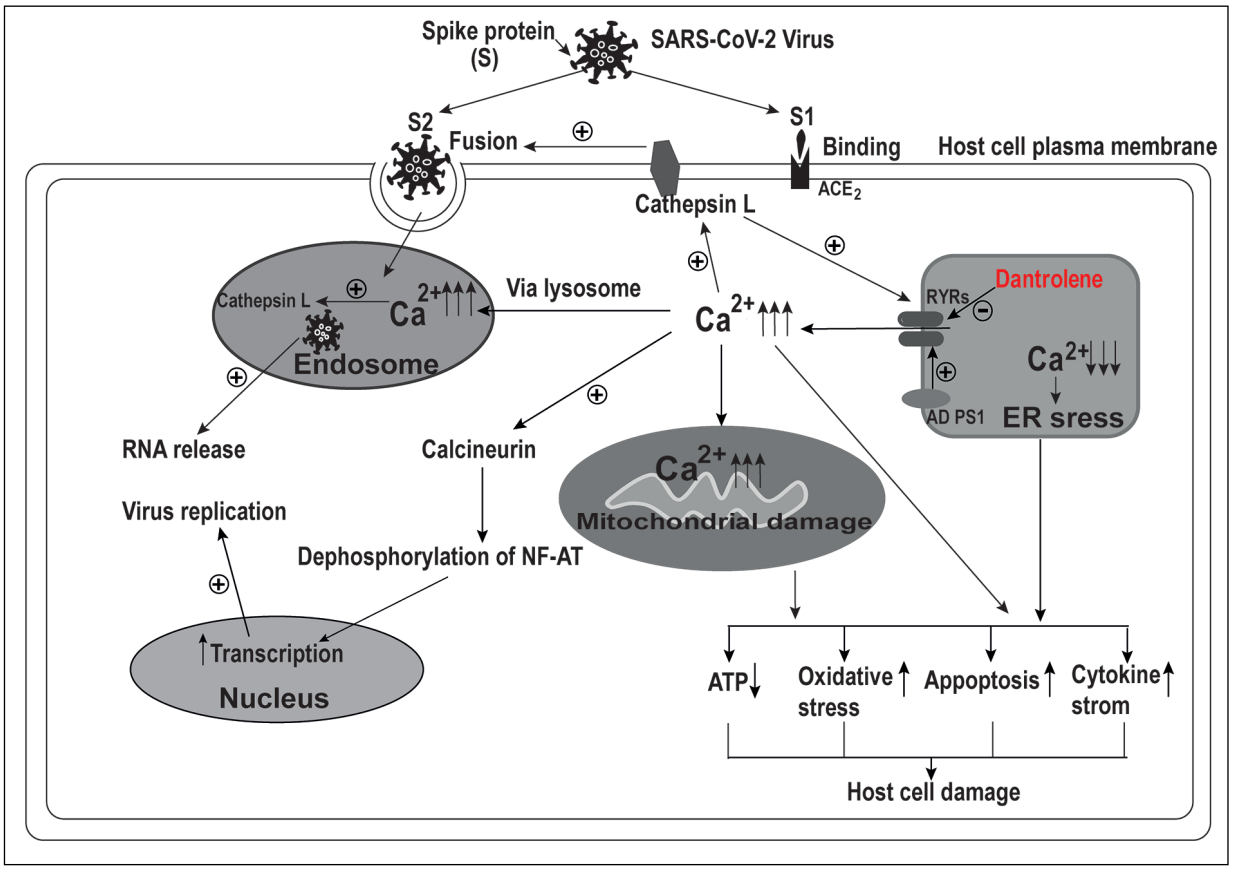

Figure 1. Dantrolene might inhibit infection and replication of SARS-CoV-2 and associated pathology. Cathepsin L, a protease on the plasma membrane of host cells, increases Ca^{2+} release from the endoplasmic reticulum (ER) via the ryanodine receptors (RyRs). The associated elevation of cytosolic Ca^{2+} concentration, in turn, increases cathepsin L activity. Cathepsin L promotes virus fusion with host cells by cleaving and activating the spike (S) protein. High levels of extracellular and cytosolic Ca^{2+} concentrations are also necessary for virus fusion and endocytosis. Cathepsin L in the endosome, under the condition of a high level of Ca^{2+} concentration, promotes virus RNA release into the cytosol. On the other hand, the increased cytosolic Ca^{2+} concentration due to the overactivation of RyRs activates calcineurin, which dephosphorylates NF-AT and translocates into the nucleus for promoting transcription and virus replication. Excess Ca^{2+} release from ER via overactivation of RyRs in AD cells results in depletion of ER Ca^{2+} and associated ER stress, as well as the overloading of mitochondria with Ca^{2+} and associated mitochondria damage. All of the above pathologies eventually result in impaired ATP production, oxidative stress damage, apoptosis, and cytokine storm, leading to final host cell damage. Dantrolene inhibits the infection and replication of the SARS-CoV-2 virus and host cell damage by inhibiting abnormal and excessive activation of RyRs and restoring the intracellular Ca^{2+} homeostasis.

liminary priming step of these enveloped viruses[14]. Meanwhile, cathepsin L promotes activation of the ryanodine receptors (RyRs)[15], which results in an abnormal increase in cytosolic Calcium ions (Ca^{2+}) concentration heightening the activity of Ca^{2+}-dependent cathepsin L[16]. Further, the endosomes containing the virus enter cytosol via endocytosis[12], and the high Ca^{2+} concentration in the mature endosome activates cathepsin L[14,17]. These processes finally release the virus RNA into the cytosol. Dantrolene inhibits the abnormal and excessive activation of RyRs and restores the intracellular Ca^{2+} homeostasis[9], which breaks the pathological feedback between the cathepsin L and the Ca^{2+} and prevents the entry of the virus.

It was suggested that S-mediated membrane fusion was Ca^{2+}-dependent (Figure 1)[18]. The Ca^{2+} binding to fusion peptides via conserved negatively charged residues are required to trigger the fusion process[18]. To promote fusion, the virus needs additional Ca^{2+} which is imported from the ER via RyRs (Figure 1) to the endosomes. So it is intriguing to note that amiodarone, a drug that blocks endosomal/lysosomal Ca^{2+} channels, inhibits SARS-CoV entry after endosomal uptake[19]. Commonly used Ca^{2+} channel blockers showed therapeutic effects in

COVID-19 patients[20]. Dantrolene may inhibit calcium influx from extracellular space and elevation of cytosolic Ca^{2+} primarily by reducing the capacitive calcium entry (CCE). The ability of dantrolene to inhibit L-type Ca^{2+} channel or NMDA glutamate receptor is not fully clear. Therefore, it is not surprising to demonstrate that SARS-CoV entry was inhibited by Ca^{2+} chelators such as BAPTA-AM at the cytosol and endosomes[18]. The critical initiation of infection and subsequent virus replication depends on the presence of Ca^{2+}, especially the intracellular Ca^{2+} concentration[14,18]. Dantrolene is expected to ameliorate SARS-CoV-2 mediated over activation of RyRs and associated disruption of intracellular Ca^{2+} homeostasis (Figure 1)[9]. These effects, in turn, are expected to inhibit the SARS-CoV-2 virus infection of the host cells. Likewise, inspiring news demonstrated the *in vitro* antiviral cytotoxicity activity of dantrolene against SARS-CoV-2, in clinically relevant concentrations and duration, with minimal cytotoxicity of dantrolene itself[8]. Furthermore, the abnormal increase in cytosolic Ca^{2+} concentration via over activation of RyRs on the ER membrane enhances the calcineurin's activity, which promotes NF-AT nucleus translocation and transcription, leading to the subsequent promotion of virus replication in the cytosol (Figure 1)[16]. So, as an antagonist of RyRs, dantrolene is theoretically expected to inhibit the replication of SARS-CoV-2, although it needs to be investigated in future studies.

2. Proposed Mechanisms of Dantrolene to Reduce Cell Stress and Damage

1) Dantrolene Reduces Pathological Inflammation

Both SARS-CoV-2 and SARS-CoV are characterized by a pathological inflammatory response. The host inflammatory response is a major cause of tissue damage and subsequent mortality. Increased inflammatory response and elevated levels of cytokines (IL-1β, IL-6, IL-8, MCP-1, IP-10, TNFα, IFNγ, et al) have been observed in patients with COVID-19, which implied potential of a cytokine storm[21-24]. In an animal study, the cytokine and IFNγ were also detected in the lungs of the SARS-CoV-2-infected animals, which suggested that SARS-CoV-2 triggered the innate immune response and the activation of inflammation[25]. Furthermore, the SARS-CoV E protein forms a Ca^{2+} permeable channel in ERGIC/Golgi membranes. The channel activity alters Ca^{2+} homeostasis within cells and boosts the activation of the NLRP3 inflammasome, which leads to the overproduction of IL-1β[26]. The development of an uncontrolled inflammatory response can thus lead to detrimental outcomes such as diffused alveolar damage and fibrosis, progressive respiratory failure, and multiple organ damage and dysfunction. Additionally, inflammation and SARS-CoV proteins cause ER stress, which consequently leads to dysregulation of Ca^{2+} homeostasis[27,28].

Intracellular Ca^{2+} signalling is essential in the release of pro-inflammatory cytokines and the elevation of the intracellular Ca^{2+} has been suggested to be a critical event in sepsis[29]. Calcium influx may play a partial role in promoting the plasma levels of cytokines, because the calcium channel blockers have been demonstrated to ameliorate excessive inflammation[30]. Subsequently, calcium channel blockers have been proposed to treat COVID-19 patients[31]. With its ability to ameliorate Ca^{2+} dysregulation by inhibiting over activation of RyRs (Figure 1), dantrolene has been demonstrated to suppress plasma and tissue concentration of IL-6[32], IL-8[33], IL-1β, TNF-α[34,35], and IFN-γ[36] *in vivo* and *in vitro*. Consequently, dantrolene inhibited ER-mediated Ca^{2+} release and ameliorated ER stress[37].

2) Dantrolene Reduces Pathological Oxidative Stress

Oxidative stress generated from SARS-CoV-2, might further exacerbate the pro-inflammatory epigenetic changes and result in a vicious circle of cytokine response. At the same time, response to SARS-CoV-2 infection, DNA methylation defect exacerbated by oxidative stress will further enhance viral entry through epigenetic de-repression of ACE2 and increased ACE2 expression[38]. As for SARS-CoV, oxidative stress-sensitive genes were upregulated in peripheral blood mononuclear cells of patients[39]. Alterations of reactive oxygen species (ROS) production that are caused by respiratory viral infections are implicated in inflammation, lung epithelial disruption, tissue damage, and even pulmonary fibrosis[40].

Given SARS-CoV induced oxidative stress cell damage, anti-oxidative treatment may play a role in the SARS-CoV treatment. Dantrolene was reported to protect cells against oxidative stress by elevating the levels of GSH and GSH/GSSG[41,42]. Calcium release from the ER was associated with the generation of ROS[43], which was inhibited by dantrolene via lowering mitochondrial superoxide, ROS[44].

3) Dantrolene Inhibits Cell Death By Apoptosis

Apoptosis is induced as one of the host antiviral responses to limit virus replication and production during viral infections. Lymphopenia was common in SARS-CoV-2 infected patients, probably due to lymphocyte apoptosis[21,24,45]. Also, laboratory research in peripheral blood mononuclear cells demonstrated that TP53, an important gene in the process of apoptosis, showed an increasing trend in patients infected with SARS-CoV-2[24]. In SARS-CoV-2 infected animals, apoptosis has been found in the respiratory tract and TUNEL staining showed the diffused signals in the lungs, bronchiolar lumen cell debris, and collapsed alveolar walls[25]. The release of Ca^{2+} from ER has been proposed to be involved in the induction of apoptosis by oxidative stress, which is also a pathological process induced by SARS-CoV-2[43].

Apoptosis contributes to SARS-CoV-2 virus pathogenesis, and inhibition of apoptosis may protect host cells against damage. Abnormal Ca^{2+} release from the ER and consequent increase in cytosolic and mitochondria Ca^{2+} levels play pivotal roles in inducing cell apoptosis in a variety of cell types[46]. Thus, dantrolene can suppress apoptosis through inhibiting RyR-mediated abnormal and excessive Ca^{2+} release[47,48]. Moreover, dantrolene can ameliorate apoptosis by directly inhibiting nuclear condensation and fragmentation[49,50].

4) Dantrolene Ameliorates Impairment of Autophagy

SARS-CoV has the potential to inhibit the autophagy process. An analysis of a relatively wide database of SARS-CoV-2 genomes of worldwide isolates representative of COVID-19 has revealed two synonymous mutations, of which one is non-structural viral proteins 6 (NSP6)[51]. NSP6 is a common component of both α and β-coronaviruses, which locates to the ER and generates autophagosomes[52]. It has been shown that NSP6 and ER binding may favor coronavirus infection by compromising the ability of autophagosomes to deliver viral components to lysosomes for degradation[53,54]. Thus, this would limit autophagosome expansion and activity[55]. Moreover, overexpression of membrane-associated papain-like protease PLP2 of SARS-CoV and MERS-CoV led to blockage of autophagosomes-lysosomes fusion and suppression of the autophagic flux[56]. It has been shown that high cytosolic Ca^{2+} concentration suppressed vesicle fusion, and calcium channel blockers can promote autophagosome-lysosome fusion[57]. Dantrolene, as a calcium channel blocker, through inhibition of the RyRs in ER, has been reported to promote autophagy activity by inducing autophagy induction[58,59] and, therefore, potentially ameliorating the impaired autophagy function mediated by SARS-CoV-2 viruses.

3. Dantrolene Potentially Ameliorates the Multiple Organ Damages in COVID-19 Patients

COVID-19 typically demonstrates severe progressive lung injury, multi-organ failure, and death[3,60,61]. Although SARS-CoV-2 initially infects the lungs and causes lung damage, the virus eventually reaches many organs, resulting in multiple organ damage[62]. Critically ill patients are typically found to have systemic multiple-organ damage and dysfunction[63,64].

1) Lung

Acute respiratory distress syndrome (ARDS) is often seen in critically ill COVID-19 patients, which is usually life-threatening because it is associated with progressive hypoxia and associated multiple organ damage[3,60,65]. Pulmonary hypertension (PH) is a recognized consequence of ARDS and a severe condition with a very poor survival rate[66,67], which was presented in COVID-19 patients[68]. Pulmonary vasoconstriction due to hypoxia and inflammation constitutes the majority of the underlying mechanisms of PH[69]. It has been proposed that the correction of abdominal pH by reducing hypoxic pulmonary vasoconstriction could benefit COVID-19 patients.

RyRs play an important role in hypoxia-induced Ca^{2+} release and contraction[70], which contributes significantly to the development of pulmonary hypertension[71]. Chronic hypoxia increases RyR2 expression and further induces pulmonary hypertension[72]. Dantrolene can inhibit hypoxia-induced Ca^{2+} release in the pulmonary arterial smooth muscle cell and vasoconstriction of the pulmonary artery[70,73,74], which reverses the hypoxic vasoconstriction[75]. In light of this beneficial effect, dantrolene may be a potential adjunctive countermeasure.

Moreover, in the airway smooth muscle, RyRs also mediate the Ca^{2+} response and thus bronchoconstriction, which can be attenuated by dan-

trolene[76]. This potentially mitigates the high airway pressure, which might result in the pneumothorax of COVID-19 patients[77].

2) Cardiovascular System

Cardiac injury in COVID-19 patients was more likely related to multiple stress factors rather than direct damage by the virus[68]. Therefore, the goal is to minimize the myocardial ischemia and ischemia-reperfusion injury (IRI) in these patients.

Cytosolic Ca^{2+} overload plays a major role in the development of irreversible injury during myocardial ischemia, while the abnormal Ca^{2+} release from the sarcoplasmic reticulum contributes to this damage significantly[78]. Dantrolene reduced ischemic injury even at concentrations that did not affect contractile performance in the heart[79]. *In vitro* studies showed that dantrolene attenuated the lethal cellular injury[80], reduced infarct damage[79-81], protected cardiac function[79,82,83], and was even antiarrhythmic[83] under IRI.

Cardiac arrhythmia and associated cardiac arrest are often seen in COVID-19 patients[3]. In heart failure, arrhythmogenic Ca^{2+} release and chronic Ca^{2+} depletion arise due to the altered function of the RyR Ca^{2+} release channel[84]. Dantrolene has been demonstrated to have antiarrhythmic effects against Ca^{2+} overload mediated arrhythmias[85,86], while at the same time preserving inotropy[84]. Dantrolene can also improve survival after ventricular fibrillation by mitigating impaired Ca^{2+} handling in animal models[87], and prevent catecholaminergic polymorphic ventricular tachycardia[88].

3) Brain

The expression and distribution of ACE2 in the brain[89] suggest that the SARS-CoV-2 may cause some neurologic manifestations through direct[90] or indirect mechanisms[91]. The infection itself has also been described as a risk factor for stroke[92]. The ischemia of the brain seems to be a severe threat to COVID-19 patients.

One approach to protect the brain against ischemia is to reduce the tissue's functional activity to preserve energy for the metabolic processes that are essential to viability[93]. The neuroprotective effect of dantrolene, which inhibits abnormal Ca^{2+} release from ER, and then contributes to the large reversible reductions in O_2 consumption, glycolysis, and electrophysiological function[93], appears rather consistent across multiple cells and animal models of neurological injury that include excitotoxicity[94-98], oxygen-glucose deprivation (OGD), forebrain ischemia[104-107], focal ischemia[108], global ischemia[109,100], and traumatic injury[111]. In humans, dantrolene is capable of attenuating cerebral vasospasm[112] and providing neuroprotection[113].

4) Liver

Many patients with COVID-19 range from differing degrees of liver damage and function abnormality[61]. Pneumonia-associated hypoxia and immune-mediated inflammation, such as cytokine storm, might contribute to liver injury or even develop into liver failure in patients who are critically ill[114].

It was reported that dantrolene offered significant functional and structural protection of the ischemic liver, by decreasing TNF-α but increasing IL-10 and was also associated with better liver function tests and less necrosis during ischemia in rat livers[115].

5) Kidney

Kidney failure may be part of whole-body events in COVID-19 patients[62]. Renal ischemia/reperfusion injury is a common cause of acute renal failure[116] and induces renal tubule apoptosis, which is associated with the elevation of the cytosolic calcium concentration[117]. The renal tubular cell injury can be attenuated by dantrolene[118].

6) Pathological Inflammation and Cytokine Storm

In COVID-19, higher plasma levels of cytokines including IL-6, IL-2, IL-7, IL-10, granulocyte-colony stimulating factor, interferon-γ–inducible protein, monocyte chemoattractant protein, macrophage inflammatory protein 1α, and TNF-α were found in ICU patients, which implied that a cytokine storm occurred[21,61]. For COVID-19 patients, cytokine storms are a major reason that some require intensive care and ventilation. Dantrolene has been shown to inhibit various cytokine release and inflammation in various animal models[34-36]. It was reported that dantrolene decreased TNF-α in the lung (26.1%), liver (29.4%), and spleen (35.4%) and IL-1α in the lung (30.0%) and liver (25.4%)[34]. These beneficial effects of dantrolene make it potentially effective at ameliorating cytokine-mediated pathological inflammatory reaction and associated cytokine storm in COVID-19 patients.

Conclusions

In such a global pandemic, little is known for certain. Besides direct antivirus treatment, attention should also be paid to reducing the severity of the symptoms, protecting the organs, and ameliorating the deterioration. Based on previous studies illustrating the dantrolene protective effects against SARS-CoV-2 virus cytotoxicity in host cells, cell or organ damage induced by hypoxia/ischemia, mitochondrial damage, oxidative stresses, inflammation, impaired autophagy function, etc., we propose that dantrolene might be a potential repurposed drug for the treatment of COVID-19 patients (Figure 2), with an expectation to assist in reducing mortality. Further studies at the varied molecular, cellular, animal, and patient levels are important and recommended.

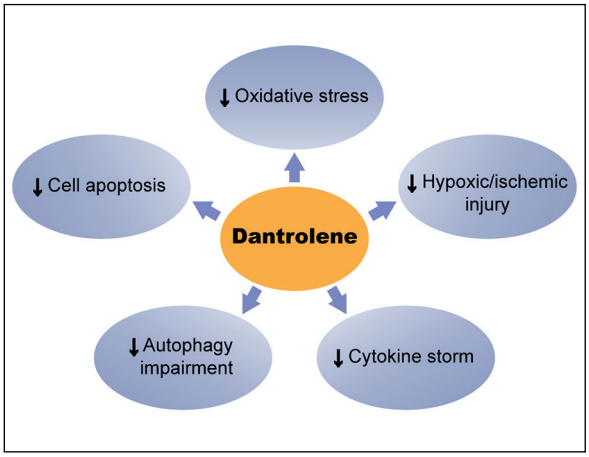

Figure 2. Dantrolene is expected to protect cell and organ damage induced by multiple pathological stresses in COVID-19 patients.

Conflict of Interest

Drs. Huafeng Wei and Ge Liang are listed as inventors of a US provisional patent application entitled "Intranasal Administration of Dantrolene for Treatment of Alzheimer's Disease" filed on June 28, 2019 (Serial number 62/868,820) by the University of Pennsylvania Trustee. The provisional patent application is also part of the research collaboration agreement between the University of Pennsylvania and Eagle Pharmaceutical Company, which produces and sells a new formula of dantrolene (Ryanodex) for the treatment of malignant hyperthermia. Other authors declare no conflict of interest.

Acknowledgements

We appreciate the English editing by Matan Ben-Abou from Drexel University, Philadelphia, PA, USA.

Funding

This work was supported by grants to HW from the National Institute on Aging (R01AG061447). The funding paid a partial salary to HW and GL and full salary to SL.

Authors' Contribution

H.W. conceived the idea. All authors contributed to the writing of this manuscript. All authors have read and approved the manuscript.

References

1) Verity R, Okell LC, Dorigatti I, Winskill P, Whittaker C, Imai N, Cuomo-Dannenburg G, Thompson H, Walker PGT, Fu H, Dighe A, Griffin JT, Baguelin M, Bhatia S, Boonyasiri A, Cori A, Cucunuba Z, FitzJohn R, Gaythorpe K, Green W, Hamlet A, Hinsley W, Laydon D, Nedjati-Gilani G, Riley S, van Elsland S, Volz E, Wang H, Wang Y, Xi X, Donnelly CA, Ghani AC, Ferguson NM. Estimates of the severity of Coronavirus disease 2019: a model-based analysis. Lancet Infect Dis 2020; 20: 669-677.

2) Wang Y, Zhang D, Du G, Du R, Zhao J, Jin Y, Fu S, Gao L, Cheng Z, Lu Q, Hu Y, Luo G, Wang K, Lu Y, Li H, Wang S, Ruan S, Yang C, Mei C, Wang Y, Ding D, Wu F, Tang X, Ye X, Ye Y, Liu B, Yang J, Yin W, Wang A, Fan G, Zhou F, Liu Z, Gu X, Xu J, Shang L, Zhang Y, Cao L, Guo T, Wan Y, Qin H, Jiang Y, Jaki T, Hayden FG, Horby PW, Cao B, Wang C. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet 2020; 395: 1569-1578.

3) Tang W, Cao Z, Han M, Wang Z, Chen J, Sun W, Wu Y, Xiao W, Liu S, Chen E, Chen W, Wang X, Yang J, Lin J, Zhao Q, Yan Y, Xie Z, Li D, Yang Y, Liu L, Qu J, Ning G, Shi G, Xie Q. Hydroxychloroquine in patients with COVID-19: an open-label, randomized, controlled trial. medRxiv 2020: 2020.04.10.20060558.

4) Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, Ruan L, Song B, Cai Y, Wei M, Li X, Xia J, Chen N, Xiang J, Yu T, Bai T, Xie X, Zhang L, Li C, Yuan Y, Chen H, Li H, Huang H, Tu S, Gong F, Liu Y, Wei Y, Dong C, Zhou F, Gu X, Xu J, Liu Z, Zhang Y, Li H, Shang L, Wang K, Li K, Zhou X, Dong X, Qu Z, Lu S, Hu X, Ruan S, Luo S, Wu J, Peng L, Cheng F, Pan L, Zou J, Jia C, Wang J, Liu X, Wang S, Wu X, Ge Q, He J, Zhan H, Qiu F, Guo L, Huang C, Jaki T, Hayden FG, Horby PW, Zhang D, Wang C. A trial of lopinavir-ritonavir in adults hospitalized with severe Covid-19. N Engl J Med 2020; 382: 1787-1799.

5) Shneider A, Kudriavtsev A. Can melatonin reduce the severity of COVID-19 pandemic? Int Rev Immunol 2020; 39: 153-162.

6) Xu X, Han M, Li T, Sun W, Wang D, Fu B, Zhou Y, Zheng X, Yang Y, Li X, Zhang X, Pan A, Wei H. Effective treatment of severe COVID-19 patients with tocilizumab. Proc Natl Acad Sci U S A 2020; 117: 10970-10975.

7) Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020; 395: 1054-1062.

8) Eagle Pharmaceuticals, Inc. Eagle pharmaceuticals announces laboratory test results demonstrating in vitro antiviral activity of RYANODEX® (Dantrolene Sodium) against coronavirus SARS-CoV-2. Available from, https://businesswire.com/news/home/20200416005156/en. [Accessed 10 May 2020].

9) Inan S, Wei H. The cytoprotective effects of dantrolene: a ryanodine receptor antagonist. Anesth Analg 2010; 111: 1400-1410.

10) Boys JA, Toledo AH, Anaya-Prado R, Lopez-Neblina F, Toledo-Pereyra LH. Effects of dantrolene on ischemia-reperfusion injury in animal models: a review of outcomes in heart, brain, liver, and kidney. J Investig Med 2010; 58: 875-882.

11) Hoffmann M, Kleine-Weber H, Pöhlmann S. A multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells. Mol Cell 2020; 78: 779- 784.e5.

12) Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, Guo L, Guo R, Chen T, Hu J, Xiang Z, Mu Z, Chen X, Chen J, Hu K, Jin Q, Wang J. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun 2020; 11: 1620.

13) Liu T, Luo S, Libby P, Shi GP. Cathepsin L-selective inhibitors: A potentially promising treatment for COVID-19 patients. Pharmacol Ther 2020; 213: 107587.

14) Millet JK, Whittaker GR. Physiological and molecular triggers for SARS-CoV membrane fusion and entry into host cells. Virology 2018; 517: 3-8.

15) Elliott EB, McCarroll D, Hasumi H, Welsh CE, Panissidi AA, Jones NG, Rossor CL, Tait A, Smith GL, Mottram JC, Morrison LJ, Loughrey CM. Trypanosoma brucei cathepsin-L increases arrhythmogenic sarcoplasmic reticulum-mediated calcium release in rat cardiomyocytes. Cardiovasc Res 2013; 100: 325-335.

16) Tanaka Y, Sato Y, Sasaki T. Suppression of coronavirus replication by cyclophilin inhibitors. Viruses 2013; 5: 1250-1260.

17) Straus MR, Tang T, Lai AL, Flegel A, Bidon M, Freed JH, Daniel S, Whittaker GR. Ca2+ ions promote fusion of middle east respiratory syndrome coronavirus with host cells and increase infectivity. J Virol 2020; 94: e00426-20.

18) Lai AL, Millet JK, Daniel S, Freed JH, Whittaker GR. The SARS-CoV fusion peptide forms an extended bipartite fusion platform that perturbs membrane order in a calcium-dependent manner. J Mol Biol 2017; 429: 3875-3892.

19) Stadler K, Ha HR, Ciminale V, Spirli C, Saletti G, Schiavon M, Bruttomesso D, Bigler L, Follath F, Pettenazzo A, Baritussio A. Amiodarone alters late endosomes and inhibits SARS coronavirus infection at a post-endosomal level. Am J Respir Cell Mol Biol 2008; 39: 142-149.

20) Solaimanzadeh I. Nifedipine and Amlodipine are associated with improved mortality and decreased risk for intubation and mechanical ventilation in elderly patients hospitalized for COVID-19. Cureus 2020; 12: e8069.

21) Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020; 395: 497-506.

22) Liu K, Chen Y, Lin R, Han K. Clinical features of COVID-19 in elderly patients: a comparison with young and middle-aged patients. J Infect 2020; 80: e14-e18.

23) Wong CK, Lam CW, Wu AK, Ip WK, Lee NL, Chan IH, Lit LC, Hui DS, Chan MH, Chung SS, Sung JJ. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. Clin Exp Immunol 2004; 136: 95-103.

24) Xiong Y, Liu Y, Cao L, Wang D, Guo M, Jiang A, Guo D, Hu W, Yang J, Tang Z, Wu H, Lin Y, Zhang M, Zhang Q, Shi M, Liu Y, Zhou Y, Lan K, Chen Y. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. Emerg Microbes Infect 2020; 9: 761-770.

25) Chan JF, Zhang AJ, Yuan S, Poon VK, Chan CC, Lee AC, Chan WM, Fan Z, Tsoi HW, Wen L, Liang R, Cao J, Chen Y, Tang K, Luo C, Cai JP, Kok KH, Chu H, Chan KH, Sridhar S, Chen Z, Chen H, To KK, Yuen KY. Simulation of the clinical and pathological manifestations of Coronavirus Disease 2019 (COVID-19) in golden Syrian hamster model: implications for disease pathogenesis and transmissibility. Clin Infect Dis. 2020 Mar 26:ciaa325. doi: 10.1093/cid/ciaa325. Epub ahead of print.

26) Nieto-Torres JL, Verdia-Baguena C, Jimenez-Guardeno JM, Regla-Nava JA, Castano-Rodriguez C, Fernandez-Delgado R, Torres J, Aguilella VM, Enjuanes L. Severe acute respiratory syndrome coronavirus E protein transports calcium ions and activates the NLRP3 inflammasome. Virology 2015; 485: 330-339.

27) Shi CS, Nabar NR, Huang NN, Kehrl JH. SARS-Coronavirus Open Reading Frame-8b triggers intracellular stress pathways and activates NLRP3 inflammasomes. Cell Death Discov 2019; 5: 101.

28) Fung TS, Liu DX. Human Coronavirus: host-pathogen interaction. Annu Rev Microbiol 2019; 73: 529-557.

29) Delano MJ, Ward PA. The immune system's role in sepsis progression, resolution, and long-term outcome. Immunol Rev 2016; 274: 330-353.

30) Kurum T, Tatli E, Yuksel M. Effects of carvedilol on plasma levels of pro-inflammatory cytokines in patients with ischemic and nonischemic dilated cardiomyopathy. Tex Heart Inst J. 2007; 34: 52-59.

31) Skayem C, Ayoub N. Carvedilol and COVID-19: a potential role in reducing infectivity and infection severity of SARS-CoV-2. Am J Med Sci 2020; 360: 300.

32) Ducreux S, Zorzato F, Müller C, Sewry C, Muntoni F, Quinlivan R, Restagno G, Girard T, Treves S. Effect of ryanodine receptor mutations on interleukin-6 release and intracellular calcium homeostasis in human myotubes from malignant hyperthermia-susceptible individuals and patients affected by central core disease. J Biol Chem 2004; 279: 43838-43846.

33) Hisatsune J, Nakayama M, Isomoto H, Kurazono H, Mukaida N, Mukhopadhyay AK, Azuma T, Yamaoka Y, Sap J, Yamasaki E, Yahiro K, Moss J, Hirayama T. Molecular characterization of Helicobacter pylori VacA induction of IL-8 in U937 cells reveals a prominent role for p38MAPK in activating transcription factor-2, cAMP response element binding protein, and NF-kappaB activation. J Immunol 2008; 180: 5017-5027.

34) Hotchkiss RS, Osborne DF, Lappas GD, Karl IE. Calcium antagonists decrease plasma and tissue concentrations of tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-1 alpha in a mouse model of endotoxin. Shock 1995; 3: 337-342.

35) Fischer DR, Sun X, Williams AB, Gang G, Pritts TA, James JH, Molloy M, Fischer JE, Paul RJ, Hasselgren PO. Dantrolene reduces serum TNFalpha and corticosterone levels and muscle calcium, calpain gene expression, and protein breakdown in septic rats. Shock 2001; 15: 200-207.

36) Németh ZH, Haskó G, Szabó C, Salzman AL, Vizi ES. Calcium channel blockers and dantrolene differentially regulate the production of interleukin-12 and interferon-gamma in endotoxemic mice. Brain Res Bull 1998; 46: 257-261.

37) Clark AL, Kanekura K, Lavagnino Z, Spears LD, Abreu D, Mahadevan J, Yagi T, Semenkovich CF, Piston DW, Urano F. Targeting cellular calcium homeostasis to prevent cytokine-mediated beta cell death. Sci Rep 2017; 7: 5611.

38) Sawalha AH, Zhao M, Coit P, Lu Q. Epigenetic dysregulation of ACE2 and interferon-regulated genes might suggest increased COVID-19 susceptibility and severity in lupus patients. Clin Immunol 2020; 215: 108410.

39) Shao H, Lan D, Duan Z, Liu Z, Min J, Zhang L, Huang J, Su J, Chen S, Xu A. Upregulation of mitochondrial gene expression in PBMC from convalescent SARS patients. J Clin Immunol 2006; 26: 546-554.

40) Khomich OA, Kochetkov SN, Bartosch B, Ivanov AV. Redox biology of respiratory viral infections. Viruses 2018; 10: 392.

41) Todorova VK, Siegel ER, Kaufmann Y, Kumarapeli A, Owen A, Wei JY, Makhoul I, Klimberg VS. Dantrolene attenuates cardiotoxicity of doxorubicin without reducing its antitumor efficacy in a breast cancer model. Transl Oncol 2020; 13: 471-480.

42) Keles I, Bozkurt MF, Aglamis E, Fidan AF, Ceylan C, Karalar M, Coban S, Denk B, Buyukokuroglu ME. Protective effects of dantrolene and methylprednisolone against spinal cord injury-induced early oxidative damage in rabbit bladder: a comparative experimental study. Adv Clin Exp Med 2019; 28: 1697-1704.

43) Lu Y-C, Lin M-L, Su H-L, Chen S-SJAr. ER-dependent Ca++-mediated cytosolic ROS as an effector for induction of mitochondrial apoptotic and ATM-JNK signal pathways in gallic acid-treated human oral cancer cells. Anticancer Res 2016; 36: 697-705.

44) Godai K, Takahashi K, Kashiwagi Y, Liu CH, Yi H, Liu S, Dong C, Lubarsky DA, Hao S. Ryanodine receptor to mitochondrial reactive oxygen species pathway plays an important role in chronic human immunodeficiency virus gp120MN-induced neuropathic pain in rats. Anesth Analg 2019; 129: 276-286.

45) Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, Xing F, Liu J, Yip CC, Poon RW, Tsoi HW, Lo SK, Chan KH, Poon VK, Chan WM, Ip JD, Cai JP, Cheng VC, Chen H, Hui CK, Yuen KY. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 2020; 395: 514-523.

46) Kaufman RJ. Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls. Genes Dev 1999; 13: 1211-1233.

47) Shin DH, Leem DG, Shin JS, Kim JI, Kim KT, Choi SY, Lee MH, Choi JH, Lee KT. Compound K induced apoptosis via endoplasmic reticulum Ca2+ release through ryanodine receptor in human lung cancer cells. J Ginseng Res 2018; 42: 165-174.

48) Martins BC, Torres BBJ, de Oliveira KM, Lavor MS, Osório CM, Fukushima FB, Rosado IR, de Melo EG. Association of riluzole and dantrolene improves significant recovery after acute spinal cord injury in rats. Spine J 2018; 18: 532-539.

49) Xu SY, Hu FY, Ren LJ, Chen L, Zhou ZQ, Zhang XJ, Li WP. Dantrolene enhances the protective effect of hypothermia on cerebral cortex neurons. Neural Regen Res 2015; 10: 1279-1285.

50) Wei H, Kang B, Wei W, Liang G, Meng QC, Li Y, Eckenhoff RG. Isoflurane and sevoflurane affect cell survival and BCL-2/BAX ratio differently. Brain Res 2005; 1037: 139-147.

51) Benvenuto D, Angeletti S, Giovanetti M, Bianchi M, Pascarella S, Cauda R, Ciccozzi M, Cassone A. Evolutionary analysis of SARS-CoV-2: how mutation of non-structural protein 6 (NSP6) could affect viral autophagy. J Infect 2020; 81: e24-e27.

52) Forni D, Cagliani R, Clerici M, Sironi M. Molecular evolution of human coronavirus genomes. Trends Microbiol 2017; 25: 35-48.

53) Zhou A, Li S, Khan FA, Zhang S. Autophagy postpones apoptotic cell death in PRRSV infection

through Bad-Beclin1 interaction. Virulence 2016; 7: 98-109.

54) Cottam EM, Whelband MC, Wileman T. Coronavirus NSP6 restricts autophagosome expansion. Autophagy 2014; 10: 1426-1441.

55) Tang JW, Cheung JL, Chu IM, Sung JJ, Peiris M, Chan PK. The large 386-nt deletion in SARS-associated coronavirus: evidence for quasispecies? J Infect Dis 2006; 194: 808-813.

56) Chen X, Wang K, Xing Y, Tu J, Yang X, Zhao Q, Li K, Chen Z. Coronavirus membrane-associated papain-like proteases induce autophagy through interacting with Beclin1 to negatively regulate antiviral innate immunity. Protein Cell 2014; 5: 912-927.

57) Park HW, Park H, Semple IA, Jang I, Ro SH, Kim M, Cazares VA, Stuenkel EL, Kim JJ, Kim JS, Lee JH. Pharmacological correction of obesity-induced autophagy arrest using calcium channel blockers. Nat Commun 2014; 5: 4834.

58) Wang Y, Liang G, Liang S, Mund R, Shi Y, Wei H. Dantrolene ameliorates impaired neurogenesis and synaptogenesis in induced pluripotent stem cell lines derived from patients with Alzheimer's disease. Anesthesiology 2020; 132: 1062-1079.

59) Chung KM, Jeong EJ, Park H, An HK, Yu SW. Mediation of autophagic cell death by type 3 ryanodine receptor (RyR3) in adult hippocampal neural stem cells. Front Cell Neurosci 2016; 10: 116.

60) Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen PY, Xiang J, Li SY, Wang JL, Liang ZJ, Peng YX, Wei L, Liu Y, Hu YH, Peng P, Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Zhong NS, China Medical Treatment Expert Group for C. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020; 382: 1708-1720.

61) Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020; 395: 507-513.

62) Wadman M, Couzin-Frankel J, Kaiser J, Matacic C. A rampage through the body. Science 2020; 368: 356-360.

63) Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, Wu Y, Zhang L, Yu Z, Fang M, Yu T, Wang Y, Pan S, Zou X, Yuan S, Shang Y. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med 2020; 8: 475-481.

64) Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, Gong W, Liu X, Liang J, Zhao Q. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. JAMA Cardiol 2020; 5: 802-810.

65) Arentz M, Yim E, Klaff L, Lokhandwala S, Riedo FX, Chong M, Lee M. Characteristics and outcomes of 21 critically ill patients with COVID-19 in Washington State. JAMA 2020; 323: 1612-1614.

66) Bull TM, Clark B, McFann K, Moss M. Pulmonary vascular dysfunction is associated with poor outcomes in patients with acute lung injury. Am J Respir Crit Care Med 2010; 182: 1123-1128.

67) Boissier F, Katsahian S, Razazi K, Thille AW, Roche-Campo F, Leon R, Vivier E, Brochard L, Vieillard-Baron A, Brun-Buisson C, Mekontso Dessap A. Prevalence and prognosis of cor pulmonale during protective ventilation for acute respiratory distress syndrome. Intensive Care Med 2013; 39: 1725-1733.

68) Deng Q, Hu B, Zhang Y, Wang H, Zhou X, Hu W, Cheng Y, Yan J, Ping H, Zhou Q. Suspected myocardial injury in patients with COVID-19: evidence from front-line clinical observation in Wuhan, China. Int J Cardiol 2020; 311: 116-121.

69) Price LC, Wort SJ. Pulmonary hypertension in ARDS: inflammation matters! Thorax 2017; 72: 396-397.

70) Zheng YM, Wang QS, Rathore R, Zhang WH, Mazurkiewicz JE, Sorrentino V, Singer HA, Kotlikoff MI, Wang YX. Type-3 ryanodine receptors mediate hypoxia-, but not neurotransmitter-induced calcium release and contraction in pulmonary artery smooth muscle cells. J Gen Physiol 2005; 125: 427-440.

71) Song T, Zheng YM, Wang YX. Cross talk between mitochondrial reactive oxygen species and sarcoplasmic reticulum calcium in pulmonary arterial smooth muscle cells. Adv Exp Med Biol 2017; 967: 289-298.

72) Dahan D, Ducret T, Quignard JF, Marthan R, Savineau JP, Esteve E. Implication of the ryanodine receptor in TRPV4-induced calcium response in pulmonary arterial smooth muscle cells from normoxic and chronically hypoxic rats. Am J Physiol Lung Cell Mol Physiol 2012; 303: L824-833.

73) Pourmahram GE, Snetkov VA, Shaifta Y, Drndarski S, Knock GA, Aaronson PI, Ward JP. Constriction of pulmonary artery by peroxide: role of Ca2+ release and PKC. Free Radic Biol Med 2008; 45: 1468-1476.

74) Becker S, Knock GA, Snetkov V, Ward JP, Aaronson PI. Role of capacitative Ca2+ entry but not Na+/Ca2+ exchange in hypoxic pulmonary vasoconstriction in rat intrapulmonary arteries. Novartis Found Symp 2006; 272: 259-268; discussion 68-79.

75) Du W, Frazier M, McMahon TJ, Eu JP. Redox activation of intracellular calcium release channels (ryanodine receptors) in the sustained phase of hypoxia-induced pulmonary vasoconstriction. Chest 2005; 128: 556s-558s.

76) Du W, Stiber JA, Rosenberg PB, Meissner G, Eu JP. Ryanodine receptors in muscarinic receptor-mediated bronchoconstriction. J Biol Chem 2005; 280: 26287-26294.

77) Yao W, Wang T, Jiang B, Gao F, Wang L, Zheng H, Xiao W, Yao S, Mei W, Chen X, Luo A, Sun L, Cook

T, Behringer E, Huitink JM, Wong DT, Lane-Fall M, McNarry AF, McGuire B, Higgs A, Shah A, Patel A, Zuo M, Ma W, Xue Z, Zhang LM, Li W, Wang Y, Hagberg C, O'Sullivan EP, Fleisher LA, Wei H, collaborators. Emergency tracheal intubation in 202 patients with COVID-19 in Wuhan, China: lessons learnt and international expert recommendations. Br J Anaesth 2020; 125: e28-e37.

78) Chen W, London R, Murphy E, Steenbergen C. Regulation of the Ca2+ gradient across the sarcoplasmic reticulum in perfused rabbit heart. A 19F nuclear magnetic resonance study. Circ Res 1998; 83: 898-907.

79) Yu G, Zucchi R, Ronca-Testoni S, Ronca G. Protection of ischemic rat heart by dantrolene, an antagonist of the sarcoplasmic reticulum calcium release channel. Basic Res Cardiol 2000; 95: 137-143.

80) Preckel B, Schlack W, Comfere T, Thamer V. Effect of dantrolene in an in vivo and in vitro model of myocardial reperfusion injury. Acta Anaesthesiol Scand 2000; 44: 194-201.

81) Zucchi R, Yu G, Ghelardoni S, Ronca F, Ronca-Testoni S. A3 adenosine receptor stimulation modulates sarcoplasmic reticulum Ca(2+) release in rat heart. Cardiovasc Res 2001; 50: 56-64.

82) Mitchell MB, Winter CB, Banerjee A, Harken AH. Inhibition of sarcoplasmic reticulum calcium release reduces myocardial stunning. J Surg Res 1993; 54: 411-417.

83) Said M, Becerra R, Palomeque J, Rinaldi G, Kaetzel MA, Diaz-Sylvester PL, Copello JA, Dedman JR, Mundina-Weilenmann C, Vittone L, Mattiazzi A. Increased intracellular Ca2+ and SR Ca2+ load contribute to arrhythmias after acidosis in rat heart. Role of Ca2+/calmodulin-dependent protein kinase II. Am J Physiol Heart Circ Physiol 2008; 295: H1669-1683.

84) Maxwell JT, Domeier TL, Blatter LA. Dantrolene prevents arrhythmogenic Ca2+ release in heart failure. Am J Physiol Heart Circ Physiol 2012; 302: H953-963.

85) Walweel K, Oo YW, Laver DR. The emerging role of calmodulin regulation of RyR2 in controlling heart rhythm, the progression of heart failure and the antiarrhythmic action of dantrolene. Clin Exp Pharmacol Physiol 2017; 44: 135-142.

86) Penttinen K, Swan H, Vanninen S, Paavola J, Lahtinen AM, Kontula K, Aalto-Setala K. Antiarrhythmic effects of dantrolene in patients with catecholaminergic polymorphic ventricular tachycardia and replication of the responses using iPSC models. PLoS One 2015; 10: e0125366.

87) Zamiri N, Masse S, Ramadeen A, Kusha M, Hu X, Azam MA, Liu J, Lai PF, Vigmond EJ, Boyle PM, Behradfar E, Al-Hesayen A, Waxman MB, Backx P, Dorian P, Nanthakumar K. Dantrolene improves survival after ventricular fibrillation by mitigating impaired calcium handling in animal models. Circulation 2014; 129: 875-885.

88) Kobayashi S, Yano M, Uchinoumi H, Suetomi T, Susa T, Ono M, Xu X, Tateishi H, Oda T, Okuda S, Doi M, Yamamoto T, Matsuzaki M. Dantrolene, a therapeutic agent for malignant hyperthermia, inhibits catecholaminergic polymorphic ventricular tachycardia in a RyR2(R2474S/+) knock-in mouse model. Circ J 2010; 74: 2579-2584.

89) Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 2004; 203: 631-637.

90) Moriguchi T, Harii N, Goto J, Harada D, Sugawara H, Takamino J, Ueno M, Sakata H, Kondo K, Myose N, Nakao A, Takeda M, Haro H, Inoue O, Suzuki-Inoue K, Kubokawa K, Ogihara S, Sasaki T, Kinouchi H, Kojin H, Ito M, Onishi H, Shimizu T, Sasaki Y, Enomoto N, Ishihara H, Furuya S, Yamamoto T, Shimada S. A first case of meningitis/encephalitis associated with SARS-coronavirus-2. Int J Infect Dis 2020; 94: 55-58.

91) Mao L, Jin H, Wang M, Hu Y, Chen S, He Q, Chang J, Hong C, Zhou Y, Wang D, Miao X, Li Y, Hu B. Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. JAMA Neurol 2020; 77: 1-9.

92) Markus HS, Brainin M. COVID-19 and stroke-A global World Stroke Organization perspective. Int J Stroke 2020; 15: 361-364.

93) Zager EL, Ames A, 3rd. Reduction of cellular energy requirements. Screening for agents that may protect against CNS ischemia. J Neurosurg 1988; 69: 568-579.

94) Duzenli S, Bakuridze K, Gepdiremen A. The effects of ruthenium red, dantrolene and nimodipine, alone or in combination, in NMDA induced neurotoxicity of cerebellar granular cell culture of rats. Toxicol In Vitro 2005; 19: 589-594.

95) Simpson PB, Challiss RA, Nahorski SR. Involvement of intracellular stores in the Ca2+ responses to N-Methyl-D-aspartate and depolarization in cerebellar granule cells. J Neurochem 1993; 61: 760-763.

96) Frandsen A, Schousboe A. Mobilization of dantrolene-sensitive intracellular calcium pools is involved in the cytotoxicity induced by quisqualate and N-methyl-D-aspartate but not by 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionate and kainate in cultured cerebral cortical neurons. Proc Natl Acad Sci U S A 1992; 89: 2590-2594.

97) Lei SZ, Zhang D, Abele AE, Lipton SA. Blockade of NMDA receptor-mediated mobilization of intracellular Ca2+ prevents neurotoxicity. Brain Res 1992; 598: 196-202.

98) Segal M, Manor D. Confocal microscopic imaging of [Ca2+]i in cultured rat hippocampal neurons following exposure to N-methyl-D-aspartate. J Physiol 1992; 448: 655-676.

99) Mitani A, Yanase H, Sakai K, Wake Y, Kataoka K. Origin of intracellular Ca2+ elevation induced by in vitro ischemia-like condition in hippocampal slices. Brain Res 1993; 601: 103-110.

100) Wang C, Nguyen HN, Maguire JL, Perry DC. Role of intracellular calcium stores in cell death from oxy-

gen-glucose deprivation in a neuronal cell line. J Cereb Blood Flow Metab 2002; 22: 206-214.

101) Mattson MP, Zhu H, Yu J, Kindy MS. Presenilin-1 mutation increases neuronal vulnerability to focal ischemia in vivo and to hypoxia and glucose deprivation in cell culture: involvement of perturbed calcium homeostasis. J Neurosci 2000; 20: 1358-1364.

102) Tasker RC, Sahota SK, Cotter FE, Williams SR. Early postischemic dantrolene-induced amelioration of poly(ADP-ribose) polymerase-related bioenergetic failure in neonatal rat brain slices. J Cereb Blood Flow Metab 1998; 18: 1346-1356.

103) Massote PD, Pinheiro AC, Fonseca CG, Prado MA, Guimaraes AL, Massensini AR, Gomez MV. Protective effect of retinal ischemia by blockers of voltage-dependent calcium channels and intracellular calcium stores. Cell Mol Neurobiol 2008; 28: 847-856.

104) Nakayama R, Yano T, Ushijima K, Abe E, Terasaki H. Effects of dantrolene on extracellular glutamate concentration and neuronal death in the rat hippocampal CA1 region subjected to transient ischemia. Anesthesiology 2002; 96: 705-710.

105) Zhang L, Andou Y, Masuda S, Mitani A, Kataoka K. Dantrolene protects against ischemic, delayed neuronal death in gerbil brain. Neurosci Lett 1993; 158: 105-108.

106) Phillis JW, Diaz FG, O'Regan MH, Pilitsis JG. Effects of immunosuppressants, calcineurin inhibition, and blockade of endoplasmic reticulum calcium channels on free fatty acid efflux from the ischemic/reperfused rat cerebral cortex. Brain Res 2002; 957: 12-24.

107) Yano T, Nakayama R, Imaizumi T, Terasaki H, Ushijima K. Dantrolene ameliorates delayed cell death and concomitant DNA fragmentation in the rat hippocampal CA1 neurons subjected to mild ischemia. Resuscitation 2001; 50: 117-125.

108) Li F, Hayashi T, Jin G, Deguchi K, Nagotani S, Nagano I, Shoji M, Chan PH, Abe K. The protective effect of dantrolene on ischemic neuronal cell death is associated with reduced expression of endoplasmic reticulum stress markers. Brain Res 2005; 1048: 59-68.

109) Wei H, Perry DC. Dantrolene is cytoprotective in two models of neuronal cell death. J Neurochem 1996; 67: 2390-2398.

110) Gwak M, Park P, Kim K, Lim K, Jeong S, Baek C, Lee J. The effects of dantrolene on hypoxic-ischemic injury in the neonatal rat brain. Anesth Analg 2008; 106: 227-233, table of contents.

111) Yoon KW, Mitchell HL, Broder LD, Brooker RW, Delisle RK. Trauma-induced neurotoxicity in rat hippocampal neurons. Stroke 1996; 27: 122-126.

112) Muehlschlegel S, Carandang R, Hall W, Kini N, Izzy S, Garland B, Ouillette C, van der Bom IM, Flood TF, Gounis MJ, Weaver JP, Barton B, Wakhloo AK. Dantrolene for cerebral vasospasm after subarachnoid haemorrhage: a randomised double blind placebo-controlled safety trial. J Neurol Neurosurg Psychiatry 2015; 86: 1029-1035.

113) Muehlschlegel S, Sims JR. Dantrolene: mechanisms of neuroprotection and possible clinical applications in the neurointensive care unit. Neurocrit Care 2009; 10: 103-115.

114) Zhang C, Shi L, Wang FS. Liver injury in COVID-19: management and challenges. Lancet Gastroenterol Hepatol 2020; 5: 428-430.

115) Lopez-Neblina F, Toledo-Pereyra LH, Toledo AH, Walsh J. Ryanodine receptor antagonism protects the ischemic liver and modulates TNF-alpha and IL-10. J Surg Res 2007; 140: 121-128.

116) Eefting F, Rensing B, Wigman J, Pannekoek WJ, Liu WM, Cramer MJ, Lips DJ, Doevendans PA. Role of apoptosis in reperfusion injury. Cardiovasc Res 2004; 61: 414-426.

117) Wu D, Chen X, Ding R, Qiao X, Shi S, Xie Y, Hong Q, Feng Z. Ischemia/reperfusion induce renal tubule apoptosis by inositol 1,4,5-trisphosphate receptor and L-type Ca2+ channel opening. Am J Nephrol 2008; 28: 487-499.

118) Yano T, Itoh Y, Kawamura E, Maeda A, Egashira N, Nishida M, Kurose H, Oishi R. Amphotericin B-induced renal tubular cell injury is mediated by Na+ Influx through ion-permeable pores and subsequent activation of mitogen-activated protein kinases and elevation of intracellular Ca2+ concentration. Antimicrob Agents Chemother 2009; 53: 1420-1426.