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Dantrolene repurposed to treat sepsis or septic shock and COVID-19 patients

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Abstract

OBJECTIVE: Disruption of intracellular Ca^{2+} homeostasis via excessive and pathological Ca^{2+} release from the endoplasmic reticulum (ER) and/or sarcoplasmic reticulum (SR) through ryanodine receptor (RyRs) Ca^{2+} channels play a critical role in the pathology of systemic inflammatory response syndrome (SIRS) and associated multiple organ dysfunction syndrome (MODS) in sepsis or septic shock. Dantrolene, a potent inhibitor of RyRs, is expected to ameliorate SIRS and MODS and decrease mortality in sepsis or septic shock patients. This review summarized the potential mechanisms of therapeutic effects of dantrolene in sepsis or septic shock at molecular, cell, and organ levels and provided suggestions and strategies for future clinical studies.

Keywords

Bacteria; Endotoxin; Virus; SARS-CoV-2; COVID-19; Infection; Calcium; Apoptosis; Systemic inflammation response syndrome (SIRS); Multiple organ dysfunction syndrome (MODS); Mortality

Introduction

Sepsis is defined as infected patients with an increase of ≥2 Sequential Organ Failure Score (SOFA) points, while septic shock is defined as refractory hypotension requiring vasopressors with concurrent hyperlactemia (>2 mmol/L) despite adequate fluid resuscitation[1]. Sepsis is the number one cause of both admission and mortality in critically ill patients in intensive care units (ICU)[2–4]. It affects 1.5 million patients in the US alone critically each year and leads to 250,000 deaths[5]. It cost public health care in the US alone 23.7 billion dollars in 2013[5,6]. The incidence of sepsis has steadily increased during the last

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Authors' Contributions

H.W. conceived the idea. HW, GL and RMV contributed to the manuscript preparation. All the authors reviewed and approved the final manuscript.

Competing Interests

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, Philadelphia, PA, USA. 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.

several decades[4]. Although the mortality of sepsis has decreased in recent years, it is still high – between 15–20%[5]. Despite the fact that the diagnosis of sepsis and septic shock has improved over the years[1], new effective drugs for the treatment of sepsis or septic shock are still urgently needed. Besides new drug development based on an increased understanding of the pathological mechanisms of sepsis or septic shock, old drugs already approved by the Food and Drug Administration (FDA) can provide faster supplies of effective drugs and quicker clinical studies[7].

Increasing evidence suggests that pathological Ca^{2+} release from the endoplasmic reticulum (ER) and/or sarcoplasmic reticulum (SR) via ryanodine receptor (RyRs) Ca^{2+} channels plays a critical role in systemic inflammation response syndrome (SIRS)[8,9] and associated multiple organ dysfunction syndrome (MODS)[10] in sepsis and septic shock. We proposed that dantrolene, an antagonist of RyRs, can be repurposed as an effective treatment for this disease[11–19]. This review summarized the potential mechanisms of dantrolene's therapeutic effects in sepsis at molecular, cell, and organ levels and provided suggestions and strategies for future clinical studies.

Disruption of intracellular Ca^{2+} homeostasis as a primary pathology in sepsis

Sepsis is commonly triggered by pathogen-associated molecular patterns (PAMP), such as bacterial endotoxin lipopolysaccharide (LPS) or SARS-CoV-2 virus, or damage-associated molecular patterns (DAMP), such as fibronectins, small fragments of hyaluronan, and even saturated fatty acids in response to cellular damage[20,21]. Infection is considered a primary cause of sepsis and associated septic shock[1]. Increasing evidence suggests that intracellular Ca^{2+} dysregulation caused by abnormal Ca^{2+} release from the ER/SR via over activation of RyRs plays a critical role in sepsis pathology and contributes to excessive pathological inflammation termed SIRS or "cytokine syndrome" as well as associated MODS and mortality[13,22–31]. As demonstrated in Figure 1, we have commonly seen PAMP (e.g., LPS or SARS-CoV-2 virus spike (S) protein), bind to Toll-Like Receptor 4 (TLR-4) and then activate the nuclear factor kappa light chain enhancer of activated B cells (NF-κB) to increase nuclear transcription and cytosol production of proinflammation cytokine proteins, such as tumor necrosis factor-alpha (TNF-α)[21,32,33]. TNF-α is able to increase the opening of Ca^{2+} channels on the ER membrane known as $InsP_3$ receptors ($InsP_3R$)[34], which in turn release Ca^{2+} from the ER into cytosol and trigger Ca^{2+} influx from extracellular space into cytosol via store-operated calcium channels (SOCCs) on the plasma membrane[35–37]. On the other hand, activation of TLR-4 also increases the endogenous agonist of ryanodine receptor (RyRs) Ca^{2+} channels on the ER membrane, Cyclic ADP-ribose (cADPR), and results in activation of RyRs[8,38], amplifying the Ca^{2+} release from the ER/SR and elevation of cytosol Ca^{2+} concentration ($[Ca^{2+}]_c$), which further activates RyRs and leads to Ca^{2+} induced Ca^{2+} release (CICR)[39]. Excessive and abnormal Ca^{2+} release from the ER/SR via RyRs will cause ER/SR Ca^{2+} depletion and ER/SR stress[40]. It also overloads mitochondria with Ca^{2+} and results in increased production of reactive oxygen species (ROS)[41] and/or reactive nitrate species (RNS)[42], further amplifying opening of RyRs[43,44]. Mitochondrial Ca^{2+} overloading and associated damage results in impaired ATP production[45], autophagy dysfunction[46], and apoptosis[47]. A combination of ER/SR stress and mitochondrial damage eventually leads to cell and/or organ damage, which results in clinical symptoms of sepsis and multiple

organ damage syndrome (MODS), and mortality (Figure 1). Additionally, the elevation of $[Ca^{2+}]_c$ activates inducible nitric oxide synthase (iNOS) and increases the production of nitric oxide (NO), contributing to pathological vasodilation and associated septic shock, MODS, and Mortality[10]. Dantrolene, an FDA approved drug for the treatment of malignant hyperthermia, inhibits RyRs channel opening and ameliorates the pathological decrease of ER/SR Ca^{2+} and the abnormal increase of cytosol and mitochondria Ca^{2+} concentrations as well as associated sepsis/septic shock pathology (Figure 1)[14,45]. Thus, dantrolene is expected to be an effective drug to treat MODS and reduce mortality during sepsis or septic shock[13,14,18].

Dantrolene inhibits SIRS during sepsis

Bacterial endotoxins and/or viruses activate TLR-4 and increase cytokine release as a defense mechanism to protect cell damage from invading PAMP (Figure 1). However, excessive and pathological cytokine release results in SIRS or cytokine storms, damaging cells/organs and leading to MODS[48–51]. Pathological inflammatory response with abnormally elevated levels of cytokines (IL-1β, IL-6, IL-8, MCP-1, IP-10, TNFα, IFN-γ, etc.) has been observed in sepsis patients and results in SIRS[3,13]. Therefore, amelioration of SIRS and associated MODS is considered a strategy to reduce the severity of sepsis or septic shock and associated mortality. As demonstrated in figure 1, excessive Ca^{2+} release from the ER/SR via RyRs and associated ER/SR stress play an important role in cytokine release and SIRS. Thus, inhibition of RyRs is expected to ameliorate SIRS during sepsis[13,24,25,27]. Dantrolene, a Food and Drug Administration (FDA) approved drug for the treatment of malignant hyperthermia, inhibits RyRs channel opening and ameliorates the pathological decrease of ER/SR Ca^{2+} as well as the increase of cytosol and mitochondria Ca^{2+} concentrations[52–54]. Thus, it is expected to ameliorate SIRS or “cytokine storms”.

Considering the important role of ER/SR stress caused by depletion of ER/SR Ca^{2+} via over activation of RyRs in the vicious cycle of activation of TLR-4 and the production of proinflammation cytokines (Figure 1), inhibition of RyRs and amelioration of ER/SR stress may break the vicious cycle of pathological production of proinflammation cytokines and associated SIRS. Dantrolene has been demonstrated to suppress plasma and tissue concentrations of IL-6[55], IL-8[56], IL-1β, TNF-α[13,22], and IFN-γ[24–27]. Dantrolene also significantly reduced mortality in sepsis animal models with SIRS[13,15,17,18,57,58], and showed no significant side effects or toxicity in a sepsis animal model[11]. On the other hand, dantrolene promoted protective antiinflammation cytokine, IL-10, in sepsis animal models[24]. The combined effects of dantrolene on suppressing pathological cytokine and promoting protective cytokine release make it a good candidate to balance host inflammation response towards a beneficial effect during sepsis.

Among the proinflammation cytokines, TNF-α plays a critical role in disrupting intracellular Ca^{2+} homeostasis. TNF-α activates $InsP_3R$[34] and then RyRs, promoting overloading of Ca^{2+} in mitochondria and increasing production of ROS/RNS, further activating RyRs and pathological Ca^{2+} release from the ER/SR (Fig. 1). Because RyRs have a Ca^{2+} channel conductance much higher than $InsP_3R$, it is considered a primary Ca^{2+} channel, which releases Ca^{2+} from the ER/SR into cytosol, especially during pathological

stresses. In accordance, dantrolene, via its inhibition of RyRs, demonstrates inhibition of TNF-α and associated SIRS or "cytokines storms" in various kinds of animal models[13,22,26]. Dantrolene has also been proposed to inhibit excessive and pathological inflammation triggered by the SARS-CoV-2 virus (Fig. 1) and has been repurposed to treat COVID-19 patients[53,59].

Dantrolene ameliorates MODS during sepsis or septic shock

MODS is commonly seen in sepsis, especially in septic shock patients. The mortality of sepsis patients has been closely associated with SIRS and MODS – higher numbers of organ injury (OI) result in increased mortality[60–62]. Clinical variables reflecting the severity of MODS, such as sequential organ failure assessment (SOFA) and quick SOFA (q SOFA), have been used to predict mortality in sepsis patients[49]. Clearly, an important strategy to reduce mortality in sepsis patients is the reduction of MODS[10]. Disruption of intracellular Ca^{2+} homeostasis, especially the pathological decrease of ER but increase of cytosol and mitochondria Ca^{2+} concentrations (Figure 1), plays a critical role in cell and then organ damage in sepsis. Inhibition or amelioration of the Ca^{2+} dysregulation is expected to minimize the cell and organ damage and dysfunction[9,12,15,18,63–65]. Furthermore, as abnormal and pathological Ca^{2+} release from the ER/SR via over activation of RyRs plays an important role in proinflammation cytokine release, ER/SR stresses, and mitochondria damage, and associated multiple pathology pathways, dantrolene is expected to inhibit these pathologies as well as associated cell or organ damage and dysfunction by inhibiting Ca^{2+} dysregulation in sepsis (Figure 1).

Dantrolene is thought to ameliorate MODS, and therefore mortality in sepsis or septic shock patients via the following proposed multiple mechanisms[13,53,64]. 1) Inhibition of cell damage induced by multiple pathologies resulting from excessive Ca^{2+} release from the ER/SR via over activation of RyRs in sepsis patients (Figure 1, mitochondria and ER damage[66–69], excessive reactive oxygen species (ROS)[70] or nitrate species (RNS), reduction of ATP production[71], impaired autophagy[72–74], apoptosis[75,76]). Dantrolene protected cells against oxidative stress by elevating the levels of GSH and GSH/GSSG[77,78]. Dantrolene lowered mitochondrial superoxide, ROS and associated mitochondria damage[79]. Dantrolene has been reported to promote autophagy activity by augmenting autophagy induction[74,80] and, therefore, potentially ameliorating autophagy impairment in sepsis. 2) Inhibition of hypoxia damage in various organs. Severe hypoxia and associated hypoxia damage is frequently seen in sepsis patients with lung damage and acute respiratory distress syndrome (ARDS) and is a significant contributing factor to cell and multiple organ injury[81]. While abnormal and excessive Ca^{2+} release from the ER/SR via over activation of RyRs plays an important role in hypoxia-mediated cell/organ damage[53,82], dantrolene has been demonstrated to be protective against hypoxia-induced cell/organ damage[43,54,83–85]. Dantrolene inhibited multiple organ damage, including lung[43] heart[28,77], brain[83], liver, and kidney damage[28]. Thus, dantrolene is expected to reduce MODS in critically ill COVID-19 patients[53] and in sepsis or septic shock patients. 3) Inhibition of ischemia damage in various organs. Septic shock, a severe form of sepsis, is commonly seen in sepsis patients. Ischemia in various organs during septic shock contributes to MODS significantly in these patients. Excessive and abnormal Ca^{2+} release from the ER/SR via RyRs overactivation and elevation

of $[Ca^{2+}]_c$ activates iNOS and abnormally increases NO production, which in turn causes vasodilation and hypotension during septic shock. Dantrolene has been demonstrated to inhibit iNOS and the production of NO in a sepsis animal model[86]. It is well known that pathological Ca^{2+} release from the ER/SR via RyRs plays an important role in ischemia-mediated cell and organ damage[25,83,87,88]. Accordingly, dantrolene, as a potent inhibitor of RyRs[89,90], protected ischemia damage in multiple organs, including CNS[83,88,91,92], heart[93], liver[26], kidney[28], lung[43], and muscle[94] damage. Pawar et al[19] demonstrated the effectiveness of dantrolene in treating high fever in sepsis patients[19].

Practical consideration of using dantrolene in sepsis patients

As dantrolene is a clinically available drug to treat patients suffering from malignant hyperthermia, neuroleptic malignant syndrome, etc., considerable experience regarding its methods of use and side effects has been achieved[95–97]. For future clinical studies of dantrolene treatment of sepsis patients, we propose the following practical points for designing clinical trials. 1) In-hospital *vs.* critically ill sepsis patients on ventilators: Despite the muscle relaxant effects of dantrolene and common clinical features of respiratory failure in sepsis patients, critically ill patients on ventilators can be recruited for initial clinical studies without concern for breathing difficulty due to the potential muscle relaxant effects of dantrolene. This is, in part, because the muscle relaxant effects of dantrolene are much weaker than those of muscle relaxants normally used during anesthesia practice (e.g., vecuronium), and typically do not affect patient breathing after a long-term use via oral administration[95]. The early use of dantrolene even before intubation and mechanical ventilation may provide early treatment of sepsis. Additionally, sepsis patients on ventilators typically receive muscle relaxants (e.g., vecuronium, etc.). So, the muscle relaxant effects of dantrolene may become a confounding factor for data analysis in clinical studies. However, a significant effect is not expected due to its weaker muscle relaxant effects at commonly used doses. 2) Ryanodex *vs.* traditional dantrolene: Because traditional dantrolene requires a high volume of solvent (20 mg/60 ml), which is not suitable for sepsis patients commonly suffering from acute respiratory distress syndrome (ARDS), Ryanodex, which requires a much lower volume of solvent (250 mg/5 ml), is expected to be a more flexible and less restricted drug to treat sepsis patients. 3) Dantrolene administration routes, dose, and duration: considering the disturbance of gastrointestinal function in sepsis patients, intravenous administration is preferred over oral administration. Compared to oral administration, an intravenous approach provides more reliable absorption and stable plasma concentration, minimizing the variance of therapeutic efficacy among studied patients. Long-term dantrolene administration via intranasal approach increased brain concentrations and durations without significant side effects in animal studies[98,99]. Intranasal administration of dantrolene can be considered for those sepsis or septic shock patients with significant CNS damage and symptoms. Treatment of sepsis patients with respiratory failure on ventilators is not as emergent as for malignant hyperthermia. Thus, we propose treatments at a relatively lower dose, but for a longer duration. Krause et al[95] suggested that a dose of less than 400 mg/day of dantrolene will be relatively safe and will not cause liver dysfunction. Also, continuous intravenous infusion of dantrolene for up to 7 days was tolerable in patients as long as the total dose was within the recommended safe margin[100]. On the other hand, an intravenous bolus injection of dantrolene every 6 hours

can be considered if IV access is a limitation, as long as the total daily doses do not cause liver toxicity. 4) Safety monitoring: as the primary side effect of dantrolene is liver toxicity, AST and ALT should be monitored daily, and dantrolene treatment should be stopped if AST/ALT is abnormally elevated. Other commonly observed side effects, such as muscle weakness, CNS depression, and/or gastrointestinal symptoms should be carefully monitored.

Dantrolene as a potential drug for the treatment of COVID-19 patients

As an infectious pathogen, SARS-CoV-2 virus is able to cause sepsis or septic shock in COVID-19 patients[101]. We have previously repurposed dantrolene to treat COVID-19 patients[53]. Dantrolene treats COVID-19 patients via the following proposed mechanisms[53]: 1) Inhibition of upper stream pathology in disrupted Ca^{2+} homeostasis induced by over activation of RyRs; 2) Inhibition of host cell infection and replication of SARS-CoV-2 virus and reduction of viral load; 3) Inhibition of SARS-CoV-2 virus-mediated cell damage by amelioration of downstream pathologies mediated by Ca^{2+} dysregulation, such as mitochondrial damage, ER stresses, and associated ROS toxicity, energy failure (reduced ATP production), excess inflammation, impaired autophagy, and apoptosis, etc.; 4) Inhibition of SARS-CoV-2 virus-mediated MODS and associated patient mortality.

A new case report demonstrated that intravenous dantrolene (20 mg, 4 times/day) for three days followed by oral dantrolene (50 mg, three times/day) in a young critically ill COVID-19 patient with MODS on ventilator treatment was associated with a decrease of body temperature, creatine kinase (CK), myoglobin, C-reactive protein (CRP), ferritin levels, quick extubation, and contributed to overall full recovery[59].

Conclusions

Dantrolene has been repurposed to treat sepsis or septic shock and COVID-19 patients by ameliorating ER stress and mitochondrial damage as well as associated multiple pathologies including SIRS through inhibiting excessive and pathological Ca^{2+} release from the ER/SR via RyRs. Dantrolene is expected to minimize MODS and reduce mortality in sepsis or septic shock and COVID-19 patients, pending future extensive and well-designed clinical studies.

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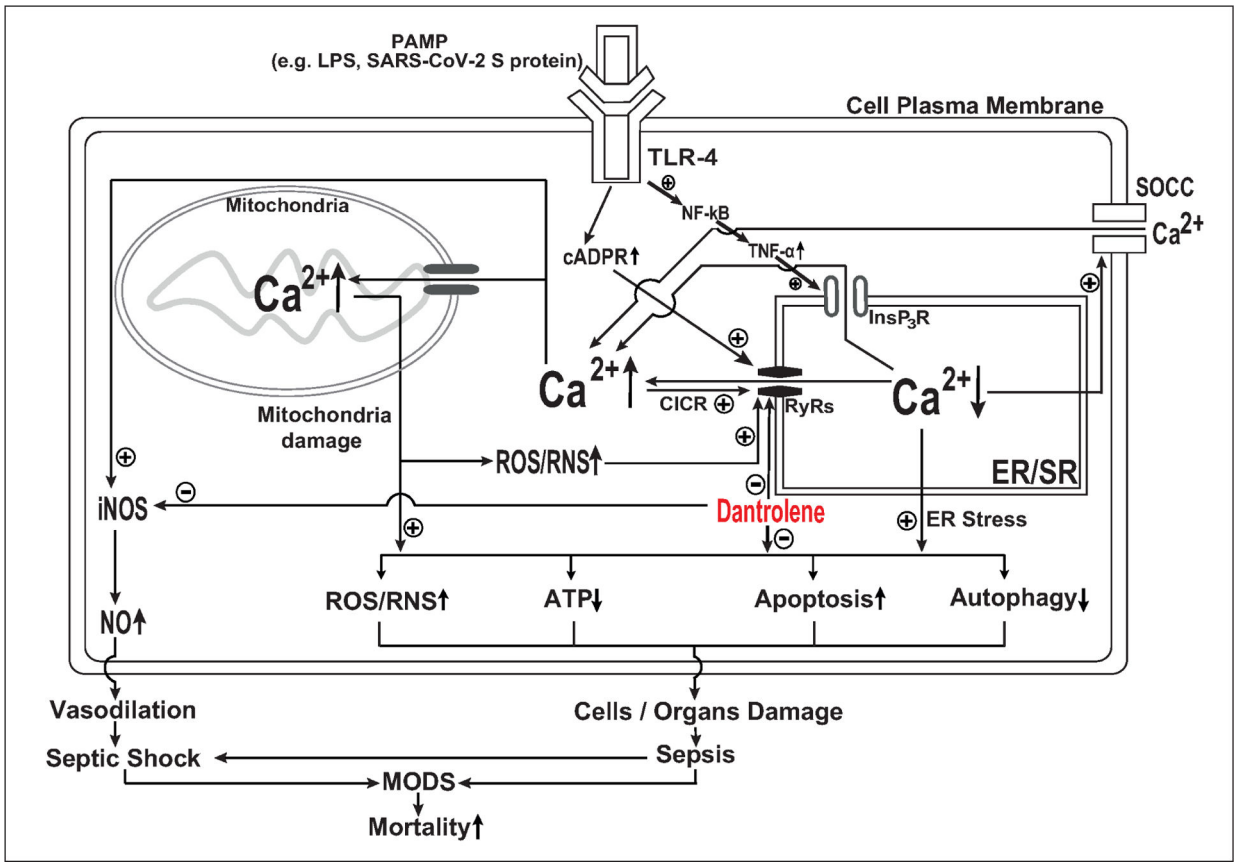

Figure 1.

Mechanisms of Dantrolene treatment of sepsis or septic shock: PAMP: pathogen-associated molecular patterns (PAMP), LPS: lipopolysaccharide (LPS), TLR-4: Toll-like receptor-4, NF-κB: activate nuclear factor kappa light chain enhancer of activated B cells, cADPR: Cyclic ADP-ribose, TNF-α: tumor necrosis factor-alpha, ER: endoplasmic reticulum, SR: sarcoplasmic reticulum, InsP3R: $InsP^3$ receptors, SOCC: store operated Ca^{2+} channels, RyRs: ryanodine receptors, CICR: Ca^{2+} induced Ca^{2+} release, ROS: reactive oxygen species, RNS: reactive nitrate species, iNOS: inducible nitric oxide synthase (iNOS), NO: nitric oxide, MODS: multiple organ damage syndrome.