Research Article

CLINICAL INVESTIGATION

Prophylactic administration of a clinically safe low dose of the COVID-19 drug candidate Rejuveinix (RJX) effectively prevents fatal cytokine storm and mitigates inflammatory organ injury in a mouse model of sepsis

Severe viral sepsis of coronavirus disease 2019 (COVID-19) is associated with a Cytokine Release Syndrome (CRS) and a high case fatality rate due to the development of Acute Respiratory Distress Syndrome (ARDS) and multi-organ failure in high-risk COVID-19 patients, including cancer patients undergoing chemotherapy. Here, we demonstrate that our COVID-19 drug candidate Rejuveinix (RJX) exhibits potent protective anti-inflammatory activity in the LPS-GalN mouse model of fatal sepsis and multi-organ failure at a dose level>10x lower than its maximum tolerated dose (MTD) for human subjects. In BALB/c mice challenged with an otherwise invariably fatal dose of LPS-GalN, prophylactic administration of 0.7 mL/kg RJX (Human equivalent dose=0.057 mL/kg), corresponding to 7.5% of its clinical Maximum Tolerated Dose (MTD), prevented the cytokine storm, mitigated the oxidative stress and inflammatory tissue injury in lungs, liver, and heart, and significantly improved the survival outcome. Furthermore, RJX increased the levels of antioxidant enzymes SOD, CAT, and GSH-Px, and reduced oxidative stress in the brain. These results indicate that RJX has clinical potential as a prophylactic anti-inflammatory agent against severe sepsis, including viral sepsis in COVID-19 patients.

Keywords: Cancer • sepsis • pneumonia • ARDS • acute lung injury • multi-organ dysfunction • cytokine release syndrome • COVID-19

Submitted: 20 January 2021; Accepted: 05 February 2021; Published online: 12 February 2021

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Introduction

Sepsis represents a strong inflammatory response to an infection with a potentially fatal outcome due to its complications, including Acute Respiratory Distress Syndrome (ARDS), septic shock, Multiple Organ Dysfunction Syndrome (MODS), and disseminated intravascular coagulopathy [1-5]. Severe viral sepsis of Coronovirus Disease 2019 (COVID-19) has a high case fatality rate due to the development of Cytokine Release Syndrome (CRS), ARDS, and multi-organ failure [6-12]. Effective prophylactic regimens capable of preventing CRS and multi-organ failure in high-risk COVID-19 patients, including those with underlying cancer requiring immunosuppressive chemotherapy, are urgently needed [6-12].

Our COVID-19 drug candidate Rejuveinix (RJX) contains several active ingredients with anti-inflammatory and anti-oxidant activity [13,14], including thiamine (Vitamin B1), riboflavin (Vitamin B2), niacinamide (Vitamin B3), pyridoxine (Vitamin B6), and ascorbic acid (Vitamin C).

Furthermore, two of its ingredients, namely thiamine and magnesium sulfate, accelerate lactate clearance, which has been shown to improve survival outcomes of patients with severe sepsis [13,14]. RJX has a very favorable clinical safety and Pharmacokinetics (PK) profile [13]. In a recently completed randomized Phase 1 study, the Maximum Tolerated Dose (MTD) of RJX in healthy volunteer subjects was determined to be 0.759 mL/kg (Protocol No. RPI003; ClinicalTrials.gov Identifier: NCT03680105) [13]. It has now entered a randomized double-blind, placebo-controlled Phase 2 study in hospitalized patients with critical COVID-19 (Protocol No. RPI015; ClinicalTrials.gov Identifier: NCT04708340).

In our preliminary studies, RJX exhibited single agent activity in a mouse model of sepsis [13]. The purpose of the present study was to further evaluate the prophylactic activity of low dose RJX, corresponding to 7.5% of its clinical MTD, in a mouse model of fatal sepsis and multi-organ failure. Our study confirms and extends our earlier work [13], thereby providing compelling preclinical proof of concept that the prophylactic use of RJX may improve the treatment outcome of severe sepsis by preventing the fatal cytokine storm and mitigating the inflammatory multi-organ injury.

Materials and Methods

LPS-GalN Model of Fatal Cytokine Storm, Sepsis, ARDS, and Multi-organ Failure

The ability of RJX to prevent fatal shock, ARDS, and multi-organ failure was examined in the well-established LPS-GalN model, as previously described [13]. In this model, LPS is combined with GalN, which further sensitizes mice to LPS-induced systemic inflammatory syndrome and multi-organ failure. The research protocol was approved by the Animal Care and Use Committee of Firat University (Project No. 04052020-391-046). Control mice were treated with 0.5 mL vehicle (i.e., Normal Saline (NS)) intraperitoneally (IP) 2 hours before and 2 hours after the IP injection of LPS-GalN. Test mice received the designated 0.7 mL/kg RJX dose (Human equivalent dose=0.057 mL/kg), corresponding to 7.5% of its clinical MTD, 2 hours before and 2 hours after the IP injection of LPS-GalN. All mice except for the untreated normal control mice were challenged with a 500 µL IP injection of LPS-GalN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine). The Kaplan-Meier method, log-rank chi-square test, was used to analyze the survival outcomes of mice in the different treatment groups. At the time of death, lungs, heart, and liver from 6 mice per group were harvested, fixed in 10% buffered formalin, and processed for histopathologic examination, as previously reported [13].

Serum inflammatory markers (IL-6, TNF-α, and LDH) were measured as reported [13]. Tissue Malondialdehyde (MDA, nmol/g tissue) levels, levels of inflammatory cytokines in tissues, and tissue activity levels of Superoxide Dismutase (SOD), Catalase (CAT), and glutathione peroxidase, as well as tissue ascorbic acid levels, were determined using previously published procedures [13].

Statistical Analyses

Standard tests were used to analyze the data sets, including Analysis of Variance (ANOVA), nonparametric analysis of variance, simple t-tests, Tukey's Test for homogeneity of variance, and pairwise tests by the Dunnett's test for parametric and nonparametric data. Tukey's multiple comparisons detected alterations among groups. The Kaplan-Meier method, log-rank chi-square test, was used to investigate survival and fatality in each group.

Results

RJX Prevents Pro-Inflammatory Cytokine Responses and Improves Survival Outcomes after LPS-GalN Induced Sepsis

Mice treated with RJX in a prophylactic setting had an improved survival outcome after being injected with LPS-GalN. In contrast to the invariably fatal treatment outcome of vehicle-treated control mice, 40% of mice treated with RJX (n=25) remained alive with a 2.4-fold longer median time survival time of 10.9 hours (Log-rank X^2=20.60; p<0.0001) (Figures 1 and 2).

In LPS-GalN challenged control mice not receiving any RJX treatments, serum IL-6, TNF-α, and LDH levels were drastically increased at the time of death, which is consistent with a "Cytokine Storm" and marked systemic inflammation (Figure 3). In contrast, RJX-treated mice that died after LPS-GalN injection had significantly lower levels of IL-6, TNF-α, as well as LDH (Figure 3), and they died much later than the vehicle-treated mice (median time to death: 5.4 hours, n=15 *vs.* 4.6 hours, n=25, p=0.0179) (Figures 1 and 2). These results collectively demonstrate that RJX decreased the pro-inflammatory cytokine responses of mice injected with LPS-GalN, and improved the survival outcome of mice post-LPS-GalN challenge.

RJX Reduces the Oxidative Stress in the Lungs and Attenuates Acute Lung Injury after LPS-GalN Induced Sepsis

When compared to untreated control mice, the lung MDA levels measuring lipid peroxidation were markedly elevated in LPS-GalN challenged mice consistent with severe oxidative stress (p<0.0001) (Figure 4). Furthermore, the tissue levels of ascorbic acid and the antioxidant enzymes SOD, CAT, and GSH-Px in the lung were markedly reduced (Figure 4). RJX significantly decreased the lung MDA levels and increased the reduced levels of the antioxidant enzymes SOD (Figure 4C), CAT (Figure 4D), GSH-Px (Figure 4E), and ascorbic acid (Figure 4A). Lungs of LPS-GalN injected mice showed histopathological evidence of severe acute lung injury. RJX mitigated the inflammatory lung

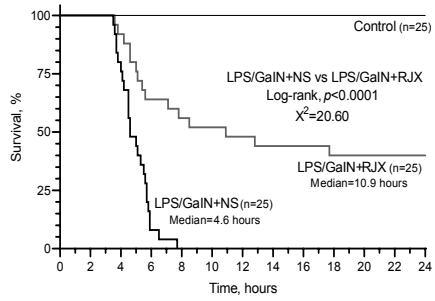

Figure 1: In Vivo Protective Activity of RJX in the LPS-GalN challenged mice. Groups of 25 BALB/c mice were treated with IP injections of 6-fold diluted RJX (4.2 mL/kg, 0.5 ml/mouse) or vehicle (NS) 2 hours before or post-injection of LPS-GalN. Except for untreated mice (Control), each mouse received 0.5 ml of LPS-GalN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) IP Survival is shown as a function of time after the LPS-GalN challenge. Depicted are the survival curves for each group along with the median survival times and the log-rank p-value for the comparison of the LPS-GalN+RJX group versus the LPS/GalN+NS group.

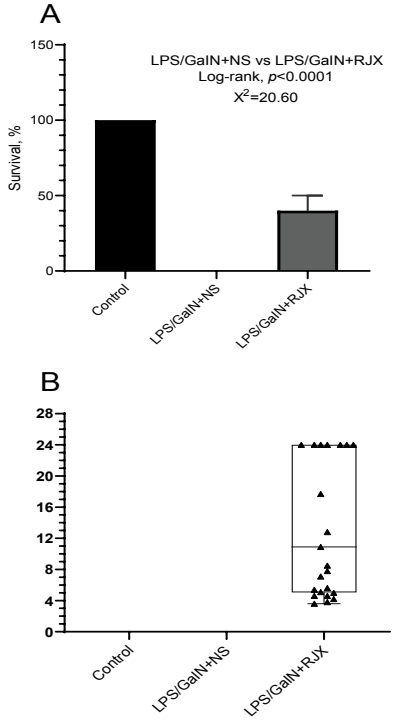

Figure 2: In Vivo Protective Activity of RJX in the LPS-GAIN challenged mice. Groups of 25 BALB/c mice were treated with IP injections of RJX (0.7 mL/kg, 0.5 ml/mouse) or vehicle (NS) 2 hours before or post-injection of LPS-GalN. Except for untreated mice (Control), each mouse received 0.5 ml of LPS-GalN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) IP Depicted are the survival data (Panel A) and time to death data (Panel B) for each group along with the median survival times and the log-rank p-value for the comparison of the LPS-GalN+RJX group versus the LPS/GalN+NS group. The time to death data for animals that were electively terminated at 24 hours was censored at 24 hours.

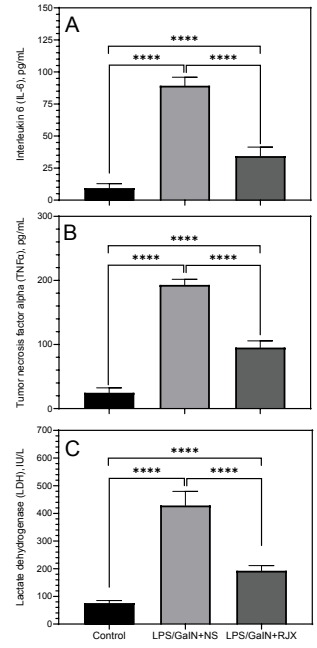

Figure 3: Effect of Rejuveinix (RJX) on Interleukin 6 (IL-6; n=19; Panel A), tumor necrosis factor-alpha (TNFα; n=14; Panel B), and Lactate dehydrogenase (LDH; n=14; Panel C) in the lipopolysaccharide-galactosamine (LPS-GalN) challenged mice. Each bar represents the mean and standard deviation for the measured laboratory parameter in the indicated specific treatment group. Groups of 25 BALB/c mice were treated with IP injections of 6-fold diluted RJX (4.2 mL/kg, 0.5 ml/mouse) or vehicle (NS) 2 hours before or post-injection of LPS-GalN. Except for untreated mice (Control), each mouse received 0.5 ml of LPS-GalN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) IP (ANOVA and Tukey's post-hoc test were used for comparing the results among different treatment groups. Statistical significance between groups is shown by ****p<0.0001).

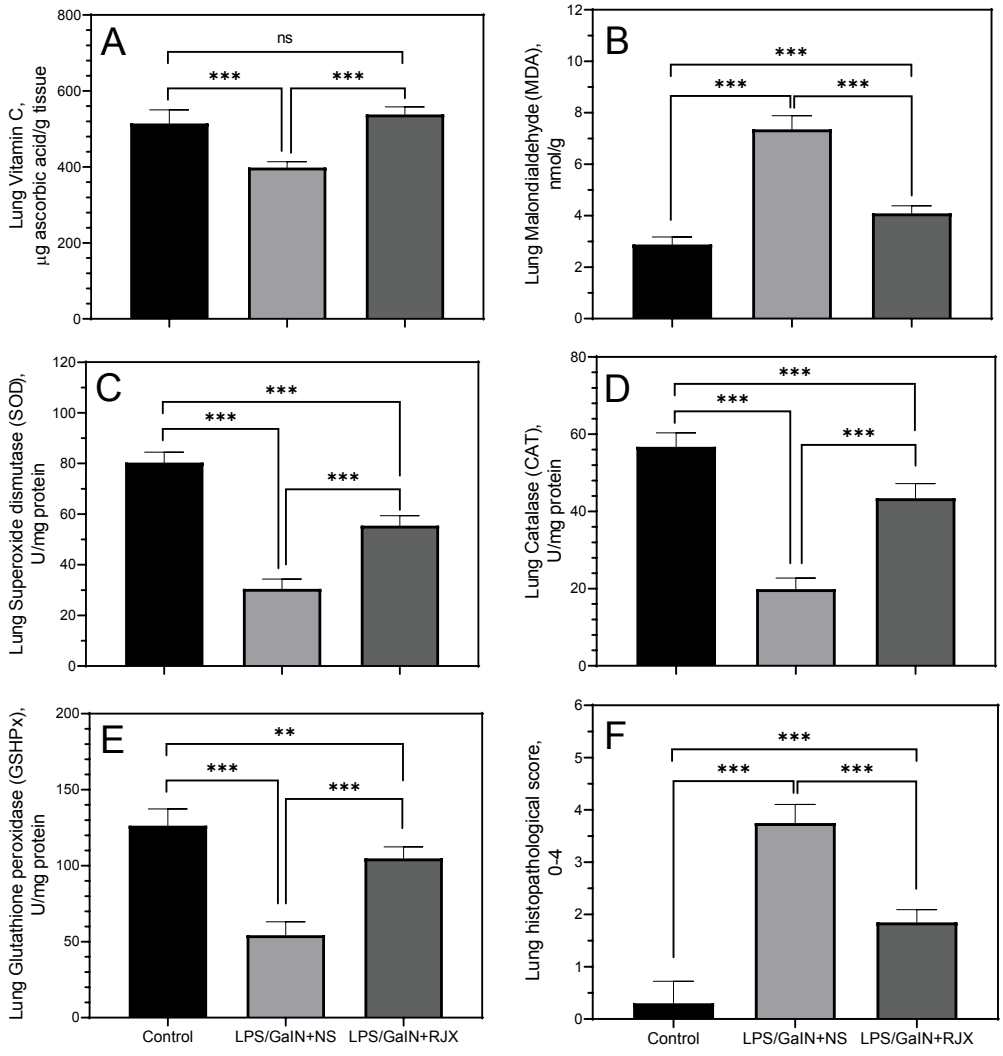

Figure 4: Tissue-Level In Vivo Anti-Inflammatory and Anti-oxidant Activity of Rejuveinix (RJX) in the LPS-GalN Mouse Model of ARDS and Multi-organ Failure. Mice (20 mice/group) were treated with IP injections of RJX (0.7 mL/kg, 0.5 ml/mouse) or vehicle (NS) 2 hours before and 2 hours post-injection of LPS-GalN. Except for untreated mice (Control), each mouse received 0.5 ml of LPS-GalN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) IP See text for discussion of results. These assays were performed on the lungs of mice that died after the LPS-GalN challenge. The lungs of the mice were harvested at the time of death which occurred within 24 hours for all mice. The depicted bars represent the mean and standard deviation for the indicated parameters in mice from the specific treatment groups. (In [A]-[E] (n=7), ANOVA and Tukey's post-hoc test were used for comparing the results among different treatment groups. Statistical significance between groups is shown by **p<0.01; ***p<0.001). In [F] (n=10), the lung histopathological score of ALI was graded according to a 5-point scale from 0 to 4 as follows: 0, I, 2, 3, and 4 represented no damage, mild damage, moderate damage, severe damage, and very severe damage, respectively. (In [F] Kruskal-Wallis test and Mann Whitney U test were used for comparing the results among different treatment groups. Statistical significance between groups is shown by ***p<0.001).

injury, as shown by the absence of alveolar hemorrhage, reduced thickening of alveolar wall, less edema/congestion, and significantly decreased leukocyte infiltration, and significantly reduced lung histopathological acute lung injury scores (Figure 4F, Figure 5).

RJX Reduces the Oxidative Stress in the Liver and Attenuates Acute Liver Damage after LPS-GalN Induced Sepsis

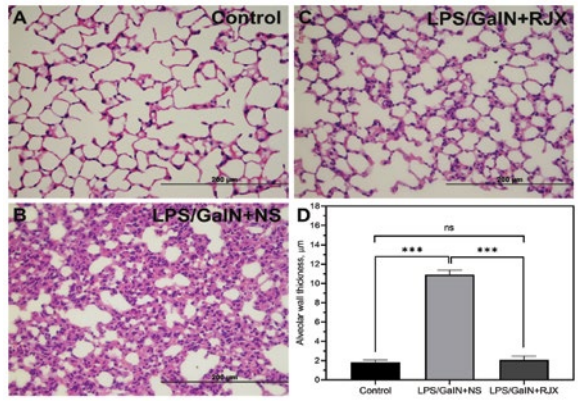

Figure 5: Prophylactic RJX Prevents Acute Lung Injury, Inflammation, and Pulmonary Edema in the LPS-GalN Mouse Model of ARDS and Multi-organ Failure. Mice were treated with IP injections of 6-fold diluted RJX (0.7 mL/kg, 0.5 ml/mouse) (Panel C) or vehicle (NS) (Panel B) 2 hours before and 2 hours post-injection of LPS-GalN. Except for untreated mice (Control) (Panel A), each mouse received 0.5 ml of LPS-GalN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) IP The RJX dose levels (in mL/kg) are indicated in parentheses. The average thickness of the alveolar wall, depicted in a bar graph representation of the mean ± SD values, was determined as a measure of pulmonary edema (Panel D) H&E X400.

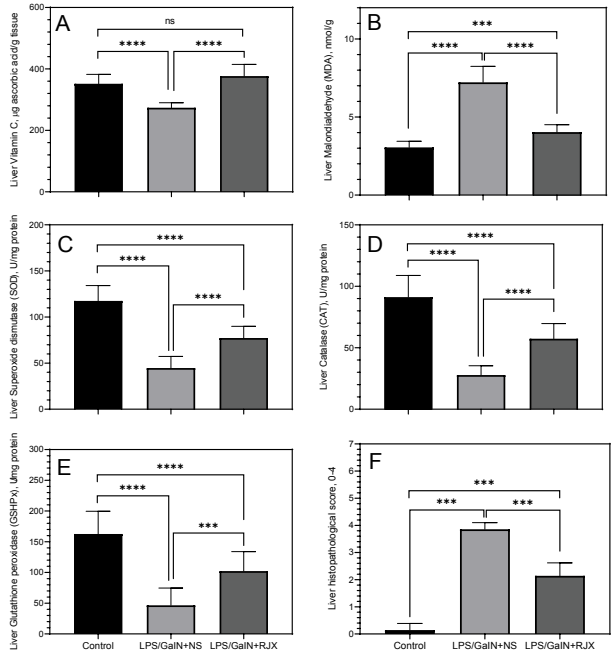

Figure 6: Effect of Rejuveinix (RJX) on liver vitamin C (Panel A, n=14), malondialdehyde (MDA; Panel B, n=19), superoxide dismutase (SOD; Panel C, n=19), catalase (CAT; Panel D, n=14), and Glutathione peroxidase (GSHPx; Panel E, n=14) and liver damage (Liver histopathological score, Panel F, n=7) in the lipopolysaccharide-galactosamine (LPS-GalN) challenged mice. Each bar represents the mean and standard deviation for the measured parameter of mice from each specific treatment group. Groups of 25 BALB/c mice were treated with IP injections of RJX (0.7 mL/kg, 0.5 ml/mouse) or vehicle 2 hours before or post-injection of LPS-GalN. Except for untreated mice (Control), each mouse received 0.5 ml of LPS-GalN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) IP (In [A]-[E] ANOVA and Tukey's post-hoc test were used for comparing the results among different treatment groups. Statistical significance between groups is shown by ***p<0.001; ****p<0.0001). In [F], the Liver histopathological score of was graded according to a 5-point scale from 0 to 4 as follows: 0, l, 2, 3, and 4 represented no damage, mild damage, moderate damage, severe damage, and very severe damage, respectively. (In [F] Kruskal-Wallis test and Mann Whitney U test were used for comparing the results among different treatment groups. Statistical significance between groups is shown by ***p<0.001).

Similar to its anti-oxidant effects in the lungs, RJX decreased the liver MDA levels (Figure 6, Panel B) and elevated the levels of SOD (Figure 6, Figure 5C), CAT (Figure 6, Figure 5D), and GSH-Px (Figure 6, Figure 5E) as well as ascorbic acid (Figure 6, Figure 5A). Further, RJX suppressed the LPS-GalN induced liver injury and inflammation, as documented by the reduction of the histopathological scores measuring liver damage (Figure 6, Figure 5F).

RJX Reduces the Oxidative Stress in the Heart and Attenuates Acute Myocardial Injury after LPS-GalN Induced Sepsis

No histopathologic lesions were observed in the heart of any of the mice challenged with LPS-GalN. However, the serum

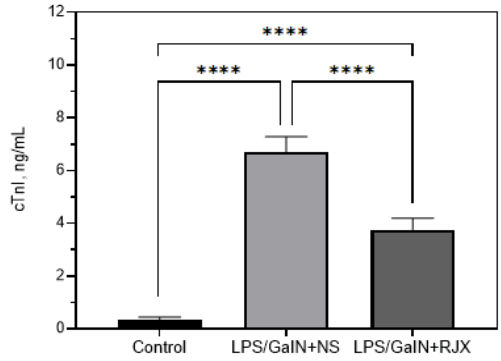

Figure 7: Effect of Rejuveinix (RJX) on serum cTnI level in LPS-GalN Mouse Model of ARDS and Multi-Organ Failure (n=7). Mice were treated with IP injections of RJX (0.7 mL/kg, 0.5 ml/mouse) or NS 2 hours before and 2 hours post-injection of LPS-GalN. Except for untreated mice (Control), each mouse received 0.5 ml of LPS-GalN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) IP The depicted bars represent the mean and standard deviation for the indicated parameters. ANOVA and Tukey's post-hoc test were used for comparing the results among different treatment groups. Statistical significance between groups is shown by: ****p<0.0001).

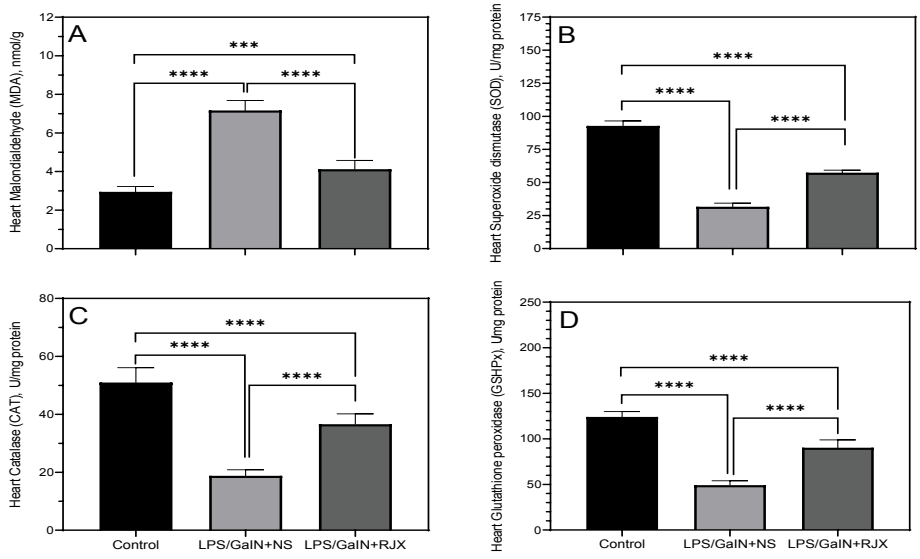

Figure 8: Heart Tissue-Level in vivo antioxidant activity of Rejuveinix (RJX) in the LPS-GalN mouse model of ARDS and multi-organ Failure (n=7). Mice were treated with IP injections of 6-fold diluted RJX (4.2 mL/kg, 0.5 ml/mouse) or vehicle (NS) 2 hours before and 2 hours post-injection of LPS-GalN. Except for untreated mice (Control), each mouse received 0.5 ml of LPS-GalN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) IP Effects of RJX on heart malondialdehyde (MDA; Panel A), superoxide dismutase (SOD; Panel B), catalase (CAT; Panel C), and glutathione peroxidase (GSHPx; Panel D) were measured in LPS-GalN challenged mice. The depicted bars represent the mean and standard deviation for the indicated parameters. ANOVA and Tukey's post-hoc test were used for comparing the results among different treatment groups. Statistical significance between groups is shown by: ***p<0.001; ****p<0.0001).

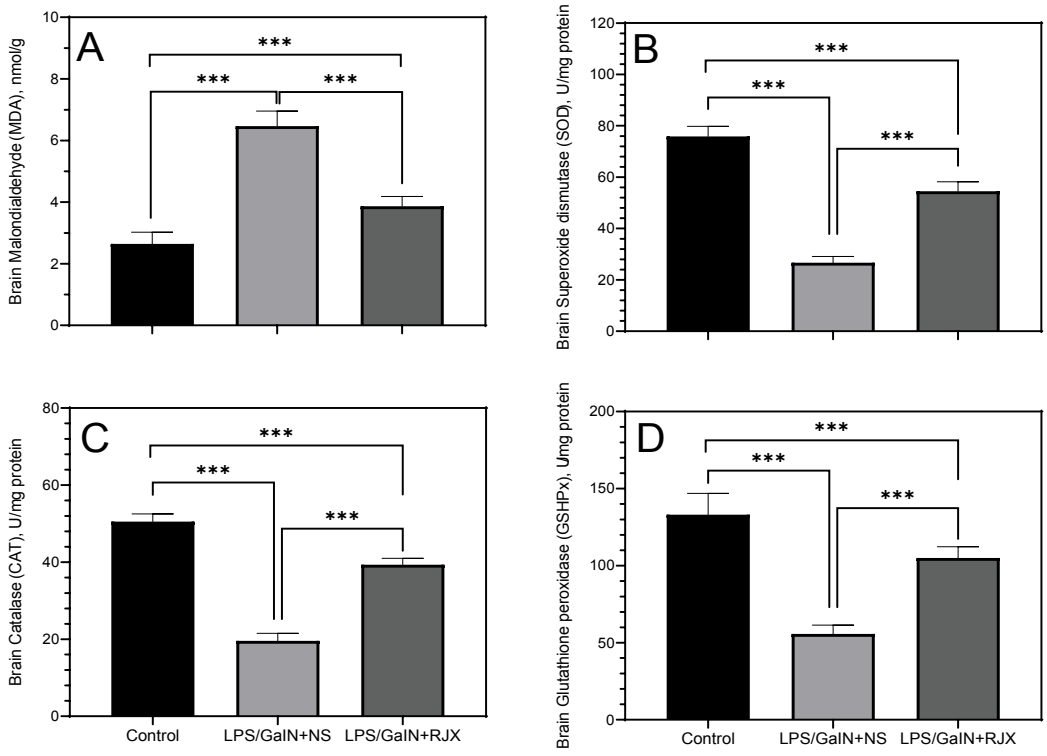

Figure 9: Effect of Rejuveinix (RJX) on brain malondialdehyde (MDA; Panel A), superoxide dismutase (SOD; Panel B), catalase (CAT; Panel C) and Glutathione peroxidase (GSHPx; Panel D) in the lipopolysaccharide-galactosamine (LPS-GalN) challenged mice. Each bar represents the mean and standard deviation for the measured parameter of mice from each specific treatment group. BALB/c mice were treated with IP injections of 6-fold diluted RJX (4.2 mL/kg, 0.5 ml/mouse) or vehicle (NS) 2 hours before or post-injection of LPS-GalN. Except for untreated mice (Control), each mouse received 0.5 ml of LPS-GalN (consisting of 100 ng of LPS plus 8 mg of D-galactosamine) IP (ANOVA and Tukey's post-hoc test. Statistical significance between groups is shown by: ***p<0.001).

Cardiac Troponin I (cTnI) levels were markedly elevated at the time of death after the LPS-GalN challenge, which is consistent with myocardial injury (Figure 7). Furthermore, the cardiac MDA levels measuring lipid peroxidation were markedly elevated, and the levels of the antioxidant enzymes SOD, CAT, and GSH-Px were markedly reduced in the heart of the LPS-GalN treated mice consistent with severe oxidative stress (Figure 8). RJX attenuated the myocardial injury as documented by a significant reduction of the serum cTnI levels (Figure 7). It also decreased the elevated MDA levels and improved the reduced levels of the antioxidant enzymes SOD, CAT, and GSH-Px, consistent with a significant reduction of oxidative stress (Figure 8).

RJX Reduces the Oxidative Stress in the Brain after LPS-GalN Induced Sepsis

No histopathologic brain lesions were observed in any of the mice challenged with LPS-GalN. However, the brain MDA levels measuring lipid peroxidation were markedly elevated, and the levels of the antioxidant enzymes SOD, CAT, and GSH-Px in the brain were markedly reduced in LPS-GalN treated mice consistent with severe oxidative stress (Figure 9). RJX decreased the brain MDA levels and normalized in a dose-dependent manner the reduced levels of the antioxidant enzymes SOD, CAT, and GSH-Px.

Discussion

Severe viral sepsis of COVID-19 is associated with CRS and a high case fatality rate due to the development of ARDS and multi-organ failure in high-risk COVID-19 patients. Here we extend our previous study [13] provide experimental evidence that RJX is capable of improving the survival outcome in the LPS-GalN mouse model of fatal sepsis. In BALB/c mice challenged with an otherwise invariably fatal dose of LPS-GalN, prophylactic administration of 0.7 mL/kg RJX (Human equivalent dose=0.057 mL/kg), corresponding to 7.5% of its clinical MTD, prevented the cytokine storm, mitigated the oxidative stress and

inflammatory tissue injury in lungs, liver, and heart, and significantly improved the survival outcome. We propose that the observed prevention of cytokine storm by the prophylactic administration of RJX combined with its anti-oxidant effects explains its ability to mitigate inflammatory injury to lungs and liver in LPS-GalN injected mice, and thereby reduce the fatality rate in the LPS-GalN mouse model of severe sepsis. Our results indicate that RJX has clinical potential as a prophylactic anti-inflammatory agent against severe sepsis, including viral sepsis in COVID-19 patients. We hypopthesize that the prophylactic use of RJX as an adjunct to standard of care will improve the treatment outcome of high-risk COVID-19 and reduce its case mortality rate.

SOD, CAT, and GSH-Px are three pivotal antioxidant defense enzymes, and their levels are altered by the level of oxidative stress that is a hallmark of severe inflammation of sepsis [14]. Oxidative stress caused by the massive production of Reactive Oxygen Species (ROS) is thought to play a major role in the pathogenesis of severe viral sepsis in COVID-19 as well [14,15]. The MDA levels were markedly elevated in the brain specimens of the LPS-GalN challenged mice which is consistent with increased lipid peroxidation. In parallel, the levels of the antioxidant enzymes SOD, CAT, and GSH-Px were profoundly suppressed due to severe oxidative stress. RJX exhibited potent anti-oxidant activity and mitigated lipid peroxidation, as reflected by significantly decreased tissue MDA levels and normalization of the tissue levels of the antioxidant enzymes SOD, CAT, and GSH-Px as well as ascorbic acid. We hypothesize that RJX will shorten the time to resolution of ARDS and viral sepsis in COVID-19 patients by preventing the development of a fulminant cytokine storm as well as reversing the cytokine-mediated multi-system inflammatory process and oxidative stress, thereby mitigating the inflammatory organ injury.

More than a third of patients with COVID-19, especially those with severe to critical COVID-19 who are treated on an Intensive Care Unit (ICU) develop Central Nervous System (CNS) symptoms and signs (e.g., headache, dizziness, ataxia, seizure, delirium, confusion, impaired consciousness) consistent with CNS involvement and /or neurological complications [16-23]. Cerebrovascular complications of viral sepsis, including ischemic or hemorrhagic stroke, CNS involvement in CRS, hypoxia as well as interplay of comorbidities have been implicated as contributing factors [16-23]. Due to the neuro-invasive capability of SARS-CoV-2, Acute Disseminated Encephalomyelitis (ADEM) and viral encephalitis have also been suspected in some patients [23]. Chaudry et al. recently proposed that reactive oxygen intermediates and oxidative stress may play an important role in the pathophysiology of COVID-19 associated CNS disease, reminiscent of their role in PD [24]. Notably, RJX increased the tissue levels of antioxidant enzymes SOD, CAT, and GSH-Px, and it reduced oxidative stress in the brain. These findings demonstrate the clinical impact potential of RJX as a neuroprotective COVID-19 and sepsis drug candidate.

Oxidative stress owing to mitochondrial dysfunction has been implicated in the pathophysiology of Alzheimer's Disease (AD) the most common form of dementia, Parkinson's Disease (PD), the second most common progressive disorder of the central nervous system, and Huntington's Disease (HD), a neurodegenerative disorder associated with cognitive decline and dementia. Notably, in a mouse CNS model of severe oxidative stress, RJX rapidly and substantially increased the levels of the antioxidant enzymes SOD, CAT, GSH-Px that were reduced in the brains of LPS-GalN treated mice consistent with the severe oxidative stress. The results presented herein demonstrate for the first time that RJX could have therapeutic utility in the treatment of AD, PD, HD, and ALS. Furthermore, because of the well-established role of oxidative stress in the development and progression of ischemic stroke, one of the leading causes of mortality and morbidity, RJX could significantly improve the standard of care for stroke as well.

Conclusion

Our results indicate that RJX has clinical potential as a prophylactic anti-inflammatory agent against severe sepsis, including viral sepsis in COVID-19 patients. We hypothesize prophylactic use of RJX as an adjunct to standard of care will improve the treatment outcome of high-risk COVID-19 and reduce its case mortality rate. Our results regarding the neuroprotective and oxidative stress mitigating effects of RJX also suggest that RJX could have therapeutic utility in the treatment of AD, PD, HD, ALS, and stroke.

Author Contributions

Each author has made significant and substantive contributions to the study, reviewed and revised the manuscript, provided final approval for submission of the final version. No medical writer was involved. F.M.U conceived the study, designed the evaluations reported in this paper, directed the data compilation and analysis, analyzed the data, and prepared the initial draft of the manuscript. Each author had access to the source data used in the analyses I.H.O. performed the necropsies and histopathologic examinations on mice.

Funding/Support

This study was funded by Reven Pharmaceuticals, LLC, a wholly-owned subsidiary of Reven Holdings Inc.

Conflicts of Interest

F.M.U., N.P., J.P. and M.V have consulting contracts with Reven Pharmaceuticals, the sponsor for the clinical development of RJX. C.O., N.S., I.H.O, and K.S declare no current competing financial interests.

Executive summary

Severe viral sepsis of Coronavirus Disease 2019 (COVID-19) is associated with a Cytokine Release Syndrome (CRS) and a high case fatality rate due to the development of ARDS and multi-organ failure in high-risk COVID-19 patients, including cancer patients undergoing chemotherapy. Here, we demonstrate that our COVID-19 drug candidate Rejuveinix (RJX) exhibits potent protective anti-inflammatory activity in the LPS-GalN mouse model of fatal sepsis and multi-organ failure at a dose level>10x lower than its Maximum Tolerated Dose (MTD) for human subjects. In BALB/c mice challenged with an otherwise invariably fatal dose of LPS-GalN, prophylactic administration of 0.7 mL/kg RJX (Human equivalent dose=0.057 mL/kg), corresponding to 7.5% of its clinical Maximum Tolerated Dose (MTD), prevented the cytokine storm, mitigated the oxidative stress and inflammatory tissue injury in lungs, liver, and heart, and significantly improved the survival outcome. Furthermore, RJX increased the levels of antioxidant enzymes SOD, CAT, and GSH-Px, and reduced oxidative stress in the brain. These results indicate that RJX has clinical potential as a prophylactic anti-inflammatory agent against severe sepsis, including viral sepsis in COVID-19 patients.

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