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Mitoquinone mesylate as post-exposure prophylaxis against SARS-CoV-2 infection in humans: an exploratory single center pragmatic open label non-randomized pilot clinical trial with matched controls

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Summary

Background An ongoing important need exists to rapidly develop novel therapeutics for COVID-19 that will retain antiviral efficacy in the setting of rapidly evolving SARS-CoV-2 variants and potential future development of resistance of SARS-COV-2 to remdesivir and protease inhibitors. To date, there is no FDA-approved treatment for post-exposure prophylaxis against SAR-CoV-2. We have shown that the mitochondrial antioxidant mitoquinone/mitoquinol mesylate (Mito-MES), a dietary supplement, has antiviral activity against SARS-CoV-2 *in vitro* and in SARS-CoV-2 infected K18-hACE2 mice.

Methods In this exploratory, pragmatic open label clinical trial (ClinicalTrials.gov identifier NCT05381454), we studied whether Mito-MES is an effective post-exposure prophylaxis treatment in people who had high-grade unmasked exposures to SARS-CoV-2 within 5 days prior to study entry. Participants were enrolled in real-world setting in Los Angeles, United States between May 1 and December 1, 2022 and were assigned to either mito-MES 20 mg daily for 14 days (n = 40) or no mito-MES (controls) (n = 40). The primary endpoint was development of SARS-CoV-2 infection based on 4 COVID-19 diagnostic tests [rapid antigen tests (RATs) or PCR] performed during the study period (14 days post exposure).

Findings Out of 40 (23 females; 57.5%) study participants who took Mito-MES, 12 (30%) developed SARS-CoV-2 infection compared to 30 of the 40 controls (75%) (difference −45.0%, 95% confidence intervals (CI): −64.5%, −25.5%). Out of 40 (19 females; 47.5%) study participants in the control group, 30 (75.0%) had at least one positive COVID-19 diagnostic test and 23 (57.5%) were symptomatic. With regards to key secondary outcomes, among symptomatic SARS-CoV-2 infections, the median duration of viral symptoms was lower in the Mito-MES group (median 3.0, 95% CI 2.75, 3.25) compared to the control group (median 5.0, 95% CI 4.0, 7.0). None of the study participants was hospitalized or required oxygen therapy. Mito-MES was well tolerated and no serious side effect was reported in any study participant.

Interpretation This work describes antiviral activity of mito-MES in humans. Mito-MES was well tolerated in our study population and attenuated transmission of SARS-CoV-2 infection. Given established safety of Mito-MES in humans, our results suggest that randomized control clinical trials of Mito-MES as post-exposure prophylaxis against SARS-CoV-2 infection are warranted.

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Keywords: Mitochondrial antioxidants; SARS-CoV-2 infection; Antiviral treatment; COVID-19; Translational research; Post-exposure prophylaxis; Clinical trials

Research in context

Evidence before this study

An ongoing important need exists to rapidly develop novel therapeutics for COVID-19 that will retain antiviral efficacy in the setting of rapidly evolving SARS-CoV-2 variants and potential future development of resistance of SARS-COV-2 to remdesivir and protease inhibitors. To date, there is no FDA-approved treatment for post-exposure prophylaxis against SAR-CoV-2. We have shown that the mitochondrial antioxidant mitoquinone/mitoquinol mesylate (Mito-MES), a dietary supplement, has antiviral activity against SARS-CoV-2 *in vitro* and in SARS-CoV-2 infected K18-hACE2 mice.

Added value of this study

This work describes antiviral activity of mito-MES in humans. Mito-MES was highly effective at preventing SARS-CoV-2 infection when given within 72 h post high-grade unmasked exposures to SARS-CoV-2.

Implications of all the available evidence

Given that Mito-MES is an over-the-counter safe diet supplement that can be immediately available in humans in the setting of high-grade exposure to SARS-CoV-2, our real-world proof-of-concept open label exploratory clinical trial, suggests that Mito-MES may represent a rapidly applicable post exposure prophylaxis treatment against development of severe SARS-CoV-2 infection.

Introduction

The SARS-CoV-2 pandemic emphasizes the urgent need to determine cellular pathways that can be targeted by novel oral antivirals that ideally would target rapidly evolving SARS-CoV-2 variants. Current oral antivirals against SARS-CoV-2 such as nirmatrelvir/ritonavir (paxlovid) and molnupiravir have limitations. Current antivirals in development against SARS-CoV-2 such as nucleoside analogues and protease inhibitors have host effects that may compromise long term safety and there may be safety concerns for long-term use in Post-Acute Sequelae of SARS-CoV-2 Infection (PASC or Long COVID). For example, PF-07321332 may involve host proteases in virus entry[1] while β-d-N4-hydroxycytidine, a metabolite of molnupiravir, may interfere with host RNA polymerases[2] and have mutagenic effects in mammalian cells.[3] The ritonavir component of paxlovid has drug interactions with widely used medications.[4] Emerging resistance of SARS-CoV-2 to nucleoside analogues such as remdesivir has been described.[5] To date, there is no FDA-approved treatment for post-exposure prophylaxis against SARS-CoV-2. Thus, there is a continued need for the development of safe oral antivirals for COVID-19.

We demonstrated that the mitochondrial antioxidant mitoquinone and/or mitoquinol mesylate (Mito-MES) has *in vitro* and *in vivo* antiviral, antiapoptotic and anti-inflammatory effects in SARS-CoV-2 infection which are mediated through the Nrf2 pathway.[6] Mito-MES had nanomolar antiviral potency against independent SARS-CoV-2 variants as well as murine coronavirus. Finally, we showed that Mito-MES treatment can drastically reduce the replication of SARS-CoV-2 *in vivo* in a mouse model of SARS-CoV-2 infection.[6] Mito-MES is a molecule that consists of a ubiquinol group found in coenzyme Q_{10} (CoQ_{10}) conjugated to a lipophilic cation (TPP).[7] CoQ_{10} is the endogenous coenzyme that transfers electrons between mitochondrial complexes and protects from lipid peroxidation.[7] Mito-MES is well tolerated and has been used as diet supplement in hundreds of thousands of humans and in 5 phase II clinical trials of 222 participants[7–12] with up to one year follow up.[9]

Thus, to obtain further proof-of-concept evidence that Mito-MES has anti-SARS-CoV-2 activity in humans, we performed an exploratory, pragmatic non-randomized open label clinical trial (ClinicalTrials.gov identifier NCT05381454) of post exposure prophylaxis (PEP) of Mito-MES, compared to no Mito-MES, to prevent development of SARS-CoV-2 infection after high-risk unmasked prolonged exposure to person with *confirmed* SARS-CoV-2 infection.

Methods

Study design

This is an exploratory, pragmatic non-randomized open label clinical trial (ClinicalTrials.gov identifier NCT05381454) to study whether Mito-MES is an effective post-exposure prophylaxis treatment in people who had high-grade unmasked exposures to SARS-CoV-2 within 5 days prior to study entry. Mito-MES has also been shown to have *in vitro* and *in vivo* activity against other respiratory viruses such as RSV.[13] Thus, the registered clinical trial is an open label exploratory clinical trial of adults that will determine the safety and efficacy of Mito-MES to prevent the development and progression of severe viral infections like COVID-19 after high-risk exposure to a person with possible respiratory viral infection such as SARS-CoV-2 infection in

persons who will receive Mito-MES compared to persons who will not receive Mito-MES (controls). This is a sub-study focusing on SARS-Co-V-2 infection. We included only study participants who had exposure to confirmed SARS-CoV-2 infection (index case). Sex equity was considered in our study and we enrolled similar number of men and women among compared groups (Table 1). The study included 2 groups: Group A (Mito-MES group) included study participants who met the inclusion criteria and agreed to take Mito-MES 20 mg daily for 14 days (n = 40) for post exposure prophylaxis against SARS-CoV-2. Group B (no Mito-MES) included study participants who met the inclusion criteria, matched the demographics of the Mito-MES group (age, sex), agreed to participate in the research study and complete questionnaires but declined to receive the intervention (mito-MES). Only one study participant per unique SARS-CoV-2 exposure was included in the study. If the same study participant had another SARS-CoV-2 exposure the same person was not enrolled twice. This study also included independent household members who had similar exposure to the same index case (confirmed SARS-CoV-2 infection) and family level clustering was considered in the study analysis. This is an exploratory open label pilot study susceptible to selection bias (see limitations in the discussion). The primary endpoint was development of SARS-CoV-2 infection based on COVID-19 diagnostic tests [rapid antigen tests (RATs) or PCR] performed during the study period (14 days post exposure).

Enrolment

Participants were enrolled in real-world setting in Los Angeles, United States between May 1 and December 1 2022. Fig. 1 shows the flow chart of the study. All enrolled study participants (n = 80) completed the study.

The inclusion criteria were: 1) Adults 18–65 years old since the safety and pharmacokinetic profiles of mito-MES has only been established in adults of this age group[7–12]; 2) High-risk exposure to index case of confirmed SARS-CoV-2 infection (based on FDA approved diagnostic PCR or rapid antigen test). High-risk

Covariates	Mito-MES (N = 40)	Control (N = 40)
Sex, % *(N)*		
Female	57.5% (23)	47.5% (19)
Male	42.5% (17)	52.5% (21)
Age, *Median (IQR)*	39.5 (34.8, 42.0)	44.0 (42.0, 46.0)
Race, % *(N)*		
White	65.0% (26)	75.0% (30)
Hispanic	27.5% (11)	20.0% (8)
Other (e.g., Asian)	7.5% (3)	5.0% (2)
Smoking, % *(N)*		
Yes	15% (6)	22.5% (9)
No	85% (34)	77.5% (31)
Household size, *Median (IQR)*	4.0 (2.8, 4.0)	4.0 (3.0, 4.0)
Number of people within household exposed to index case, *Median (IQR)*	3.0 (1.8, 3.0)	2.0 (2.0, 3.0)
Immunization status, % *(N)*		
Vaccinated with COVID-19 mRNA vaccine within 6 months prior to exposure	32.5% (13)	45.0% (18)
Vaccinated with COVID-19 mRNA vaccine >6 months prior to exposure and with no booster	47.5% (19)	55.0% (22)
Unvaccinated	20.0% (8)	0% (0)
Comorbidities, % *(N)*		
None	65.0% (26)	65.0% (26)
Hypertension	15.0% (6)	20.0% (8)
Diabetes	10.0% (4)	10.0% (4)
Obesity	17.5% (7)	20.0% (8)
Cancer	5.0% (2)	0% (0)
Major immunosuppression	10.0% (4)	0% (0)
Metabolic syndrome	7.5% (3)	2.5% (1)
Other	5.0% (2)	10.0% (4)
High risk, % *(N)*		
Yes	20.0% (8)	20.0% (8)
No	80.0% (32)	80.0% (32)

IQR = Interquartile range showing 25th and 75th percentile values.

Table 1: **Characteristics of study participants in compared groups (Mito-MES versus control).**

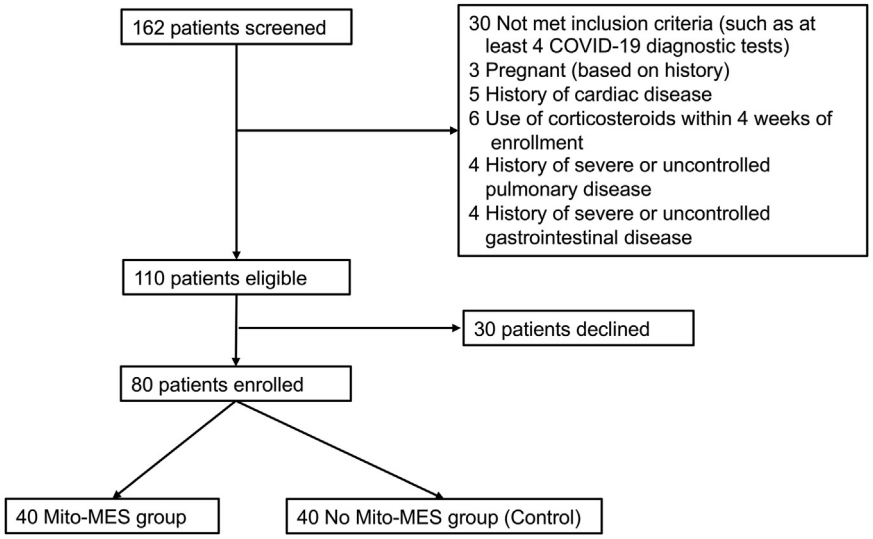

Fig. 1: **Study flow chart.**

exposure was defined as prolonged (>24 h) and intimate (<6 feet) exposure to index case *without personal protective equipment* (e.g., *face mask*) in *poorly ventilated indoor* areas.

We included both unvaccinated and vaccinated persons with or without boosters and regardless of history of prior SARS-CoV-2 infection. Although genotyping was not performed to determine the type of SARS-CoV-2 variant, emerging SARS-CoV-2 variants such as B.1.1.529, BA.2.12.1, BA.4 and BA.5 escape prior immunity from prior SARS-CoV-2 infection or vaccines.[14]

All study participants were consented, recruited and monitored by an infectious diseases physician (TK).

Outcomes

The primary endpoints were 1) to evaluate if treatment with Mito-MES can prevent the development of SARS-CoV-2 infection (defined as a positive FDA-approved SARS-CoV-2 diagnostic test) over the study period of 14 days; 2) to evaluate if treatment with Mito-MES can prevent the development of severe SARS-CoV-2 infection over the study period of 14 days. Severity of viral illness was determined based on a quantitative score system of 14 symptoms of viral illness: Symptom 1: fever, Symptom 2: cough, Symptom 3: coryza, Symptom 4: sore throat, Symptom 5: shortness of breath, Symptom 6: chills, Symptom 7: fatigue, Symptom 8: loss of smell or taste, Symptom 9: myalgias, Symptom 10: arthralgias, Symptom 11: headache, Symptom 12: nausea, Symptom 13: vomiting, Symptom 14: diarrhea. Each of 14 symptoms was given a score based on severity: 1 for mild, 2 for moderate, 3 for severe. Then a total severity score was estimated (range of score is 0–42). Presence of new onset hypoxia added an additional score of 6. Patient reported outcomes are subjective, include symptoms like fatigue that can be non-specific and definition of mild symptomatic viral illness can be variable between different persons. Thus, a stringent definition of mild viral illness was utilized to assess severity of viral illness. Mild infection was defined as a severity score $\leqq 6$ with presence of 4 or less symptoms (including fever, chills, myalgias, fatigue, cough, coryza, sore throat) *without* new onset shortness of breath and *without* new onset hypoxia. Moderate infection was defined as presence of 5 or more symptoms *with or without* shortness of breath *and/or* a severity score $\geqq 7$ (2 or more symptoms of *moderate* severity and a score of 2 per symptom) *and/or* presence of new onset hypoxia (<97% pulse oximetry). Severe infection was defined as hypoxia <92% pulse oximetry (SpO2), need for prolonged hospitalization (>2 days) or death during the study period; (3) Duration (in days) of symptoms of viral illness.

Procedures

The schedule of events for the study participants is shown in Supplementary Table S1.

Masking, allocation (intervention)

This was an open label pilot study. The control group did not receive Mito-MES or other treatment for COVID-19. The investigational drug was GMP-manufactured, is commercially available and was purchased from MitoQ Ltd. Mito-MES was provided to all study participants immediately after informed consent so that it would be available for immediate use after anticipated exposure to confirmed case of SARS-CoV-2. All study participants received 20 mg of Mito-MES (4 capsules of 5 mg each) taken orally daily after overnight fasting for 14 days post exposure to index case with SARS-CoV-2 infection. A dose of 20 mg orally daily taken after overnight fasting was chosen (not 10 mg daily) to increase absorption

based on previously established pharmacokinetics of Mito-MES used in the setting of randomized control clinical trials.[7,10] A detailed description of the pharmacokinetics of Mito-MES in blood and tissues is provided in the Supplementary Material.[7,13,15–17]

We utilized the maximum duration of treatment of Mito-MES for post-exposure prophylaxis (14 days and not 5 days). In most studies of COVID-19, treatments are given for 5–14 days.[18,19] Given emerging evidence about rebound COVID-19 after a 5-day course of Paxlovid,[20] we used the maximum possible duration of treatment for COVID-19 (14 days) so that if we find lack of efficacy in this study this can be attributed to lack of efficacy of the antiviral and not due to short duration of treatment. Notably, in many exposures there was recurrent possible high-grade exposure to SARS-CoV-2 given that people with confirmed SARS-CoV-2 infection remained in the same household like the study participants *without any use of masks* throughout the study period (14 days).

Adherence was recorded based on questionnaires and the number of pills left in the bottle at the end of the study period.

Questionnaires/patient reported outcomes

Patients were assessed by phone on days 1, 7 and 14, with a safety follow-up phone call on day 21. SARS-CoV-2 symptoms were self-reported daily from study entry to the final study visit, using the FDA guidance on Assessing COVID-19-Related Symptoms in Outpatient Adult and Adolescent Subjects in Clinical Trials of Drugs and Biological Products for COVID-19.[21] Patient-reported outcome (PRO) assessments of COVID-19-related symptoms were done at least every 24 h and were conducted at the same time each day; individual, domain, and total scores were calculated. Severity of viral illness was determined based on a quantitative score system. All study participants recorded their symptoms and number of Mito-MES pills that were taken daily in diaries that were approved by the UCLA IRB.

Other procedures

Performance of FDA-approved SARS-CoV-2 diagnostic tests was not done in a standardized manner between study participants. All nasopharyngeal (NP) swabs or midnasal swabs and FDA-approved SARS-CoV-2 PCR or RATs were done in a real-world setting (clinical care or public health resources) and outside the study outside of the trial. Performance of FDA-approved SARS-CoV-2 PCR tests and rapid antigen tests (RATs) was not done in a standardized manner between study participants and variable types of tests and number of tests were performed per study participant. This was a real-world study and the number of performed SARS-CoV-2 diagnostic tests depended on voluntary decision of each study participant based on the perceived individualized risk for SARS-CoV-2 infection. Results from the diagnostic tests (including PCR tests) were available to the investigators. Nasopharyngeal (NP) swabs were obtained from the posterior nasopharynx. Midnasal swabs were performed in both nostrils. No oral swab was performed. The utilized SARS-CoV-2 PCR and RATs diagnostic assays approved by FDA under emergency use authorization (EUA) are described in the Supplementary Material. All our study participants who had RATs had at least 3 RATs. All study participants recorded their temperature daily in diaries that were approved by the UCLA IRB. The detailed study protocol is described in the Supplementary Material.

Statistics

The study was an exploratory open label clinical trial and no formal sample size analysis was performed prior to initiation of the study. The sample size was 40 participants per group based on the number of participants that were allowed to enrol in the setting of institutional approvals and the IND. The goal of this exploratory pilot study was to collect data for sample size calculations of a future randomized control clinical trial (RCT). Key epidemiologic parameters among exposed contacts including exposure histories, cluster size, time to first positive test were analysed using descriptive statistics. In the figures, point estimates and 95% confidence intervals are presented for comparisons between treatment groups and controls. Categorical variables were recorded as the proportion of study participants with an outcome of interest (such as development of moderate viral disease yes or no) and were compared between groups using the Two sample proportion test. Continuous variables were described using medians and interquartile ranges for skewed continuous outcome of interest (such as duration of viral symptoms and severity scores). Bivariate differences were compared between groups using Wilcoxon rank sum test and the 95% confidence intervals were calculated using bootstrap method (N = 10,000 repetitions) with bias correction. Sensitivity analysis examines positive SARS-CoV-2 infection using a Generalized Estimating Equation (GEE) with family level clustering for binomial family with logit linkage. For continuous outcomes (e.g., severity scores) we utilized a GEE with for a Gaussian family. Models were fitted using exchangeable variance covariance matrix. Models were initially fitted unadjusted. Then multivariable models adjusted for key characteristics of both contacts (age, sex, co-morbidity, exposure characteristics). Covariates were included independent of each other (i.e., only one covariate in the model at a time) in order to prevent overfitting due to the small sample sizes. Similar models were used to compare the secondary outcomes between study arms. Two-sided p values less than 0.05 were considered statistically significant. All analysis conducted in R version 4.3.1 (2023-06-16 ucrt).

Sample size

Power/sample size

The study is purely exploratory and is not powered to do a formal hypothesis testing. The data from this study will set the basis for large randomized controlled clinical trials to test the safety of mito-MES in the setting of treatment of viral illnesses. Based on resources and institutional approvals, we enrolled 80 study participants.

Study approval

The trial protocol was approved by the institutional review board at University of California Los Angeles (UCLA) (IRB#21–001940). An Investigational New Drug Application (IND) was obtained by the Food and Drug Administration (FDA) to authorize administration of Mito-MES as an investigational drug (not as diet supplement) and antiviral treatment to humans. This investigational new drug (IND) study received a "Study May Proceed" letter from the FDA on April 2022. All study participants provided written informed consent. The study was conducted according to the Helsinki declaration.

Role of the funding source

The funders had no role in data collection or analysis or preparation of this manuscript. All authors had access to the dataset and take responsibility for the content and submission of this manuscript.

Results

Baseline characteristics of study participants

Participants were enrolled in real-world setting in Los Angeles, United States between May 1 and December 1 2022. Fig. 1 shows the flow chart of the study. Characteristics of study participants (n = 80) are shown in Table 1. Eight (20.0%) participants in the Mito-MES group and 8 (20.0%) participants in the control group were at high risk for severe COVID-19 (with either one major immunosuppression or at least 2 comorbidities). Four people (10.0%) in the Mito-MES group had major immunosuppression. One person had chronic myelogenous leukemia (CML) in incomplete remission on tyrosine kinase inhibitors. One person had lymphoma in complete remission on protein kinase inhibitor. Two persons had HIV with suppressed plasma viremia <50 copies/ml on antiviral treatment with CD4 T cell count >500 cells/mm^3. The participants with CML and lymphoma were unvaccinated at the time of the high-grade exposure to infected people within the household.

The study design is shown in Fig. 2. All study participants had high-grade exposures to SARS-CoV-2 defined as prolonged (>24 h) and intimate (<6 feet) exposure to index case *without masks* in *poorly ventilated indoor* areas. Twenty-four exposures in the Mito-MES group (60.0%, 12 clusters of 2 parents in each cluster) and twenty (50.0%, 10 clusters of 2 parents in each cluster) exposures in the control group included exposures of parents to infected children (who also infected their siblings) and included an *extended* window of recurrent and continuous high-risk exposure to independent confirmed SARS-CoV-2 infections within the household. In these particularly high-risk exposures, there was direct contact with children during childcare and while the children were symptomatic (fever, cough, coryza, fatigue). Children who initially got infected transmitted SARS-CoV-2 to other siblings within the family who did not get Mito-MES, confirming within household transmission *in the absence of Mito-MES use*. Each person with an initial negative SARS-CoV-2 diagnostic test [PCR or rapid antigen tests (RAT)] performed *at least* 4 SARS-CoV-2 diagnostic tests (range 4–10) in total over a period of 21 days post exposure to rule out a false negative test (Figs. 3–5). High risk study participants with particularly high-risk exposures (such as unvaccinated or immunocompromised parents exposed to their infected children) tended to perform more SARS-CoV-2 PCR diagnostic tests (up to 7 over 21 days) compared to RATs.

Mito-MES was provided to all study participants immediately after informed consent, so that it would be available for *immediate* use after *anticipated* exposure to confirmed case of SARS-CoV-2 infection. Out of 40 exposures in 40 participants in the Mito-MES group, Mito-MES was given within 24 h in 22 (55%) exposures and within 48 h in 4 (10%) exposures. In 14 (35%) cases the exact day of exposure to SARS-CoV-2 could not be reliably identified based on history and Mito-MES was started within *3–5 days* post SARS-CoV-2 infection. Twelve (30.0%) participants in the Mito-MES group took Mito-MES for 7 days instead of 14 days. Eight of these 12 participants (66.7%) initiated Mito-MES within 24 h post exposure. As per enrolment criteria, none of the study participants took other antiviral treatments for SARS-CoV-2 such as Nirmatrelvir/ritonavir.

Mito-MES safety.

One study participant in the Mito-MES group reported indigestion and heartburn during the first day that Mito-MES was started and in the setting of eating spicy food. These symptoms self-resolved after one day. The study participant successfully completed the 14-day treatment without any other side effects. No side effect was reported in any other study participant.

Mito-MES prevents SARS-CoV-2 infection in humans after high-risk exposure to SARS-CoV-2 when given within 72 h post exposure.

Despite high-grade exposures without masks, *all* diagnostic tests in *all* participants who took Mito-MES *within 72 h* (n = 26) were negative (Fig. 3) showing that *none* of the study participants developed SARS-CoV-2 infection. Two out of 14 participants (14.3%) who took Mito-MES within 3–5 days post exposure also had at least 4 negative COVID-19 diagnostic tests (Fig. 4). Thirty (75.0%) out of 40 study participants in the control

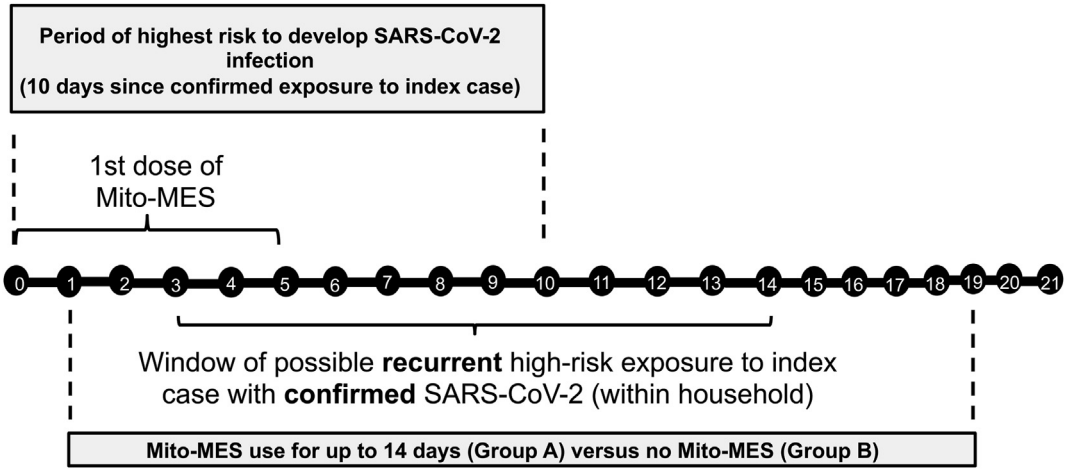

***Fig. 2:* Study design.** Study design of an open label pragmatic clinical trial of Mito-MES as post-exposure prophylaxis to prevent development of SARS-CoV-2 infection after high-risk exposure to person with confirmed SARS-CoV-2 infection. Group A included 40 people who took mito-MES (20 mg daily) for up to 14 days within 5 days post exposure (dpe). Group B included 40 people who did not take mito-MES after high-risk exposure to SARS-CoV-2. To be included in the study, all study participants were required to have *at least* 4 SARS-CoV-2 diagnostic tests [PCR in red or rapid antigen test (RAT) in green] if the initial diagnostic tests were negative. Thus, group B tended to include people with symptomatic SARS-CoV-2 infection (asymptomatic people in real-world setting do not typically do > 4 SARS-CoV-2 diagnostic tests). Diagram that illustrates time periods of intervention (Mito-MES versus no Mito-MES) and highest risk to development of SARS-CoV-2 infection with regards to day of exposure (Day 0).

group who had high-grade exposures to SARS-CoV-2 without masks, developed confirmed SARS-CoV-2 infection (Fig. 5). Eleven participants (27.5%) remained asymptomatic and had 4 serial negative SARS-CoV-2 RATs. Four participants (10.0%) developed symptoms of viral illness but had 4 serial negative SARS-CoV-2 RATs. Given limitations of RATs such as limited sensitivity,[22] false negative testing could not definitely be ruled out without SARS-CoV-2 PCR test in these 10 cases. Twelve (30.0%) participants in the Mito-MES group had a positive SARS-CoV-2 diagnostic test compared to 30 (75.0%) participants in the control group (Table 2). The odds ratio of a positive SARS-CoV-2 diagnostic test during the study period (14 days) in the Mito-MES was >80% less compared to the control group regardless of adjustment for age, sex, smoking status, high risk status and immunization status (Fig. 6). There was no reliable difference in the number of days to first positive test between the two compared groups (Table 2). Unadjusted models showed that those who received Mito-MES were statistically significantly less likely to test positive for COVID-19 on diagnostic test (Odds Ratio [OR] = 0.17). These results were robust in adjusted models which controlled for vaccination status, high risk status, smoking history, age, and sex (Supplementary Table S2). Because of the small sample sizes, these covariates were adjusted for in separate models such that only one covariate was used at a time. Similar results were found for the severity score, showing that those on Mito-MES scored 1.91 points lower in severity compared to controls (Supplementary Table S3). Overall, there was no reliable difference in the odds ratio of a positive SARS-CoV-2 diagnostic test in study participants who took Mito-MES for 7 days compared to 14 days regardless of vaccination status, high risk status, age, and sex (Supplementary Table S4).

Mito-MES reduces severity of SARS-CoV-2 infection in humans after high-risk exposure to SARS-CoV-2 when given 3–5 days post exposure.

Out of the 14 participants who started Mito-MES within *3–5 days* post SARS-CoV-2 exposure, 10 (25.0%) developed asymptomatic SARS-CoV-2 infection. Four (10.0%) developed mildly symptomatic SARS-CoV-2 infection (Fig. 4). Out of 40 study participants in the control group, 23 (57.5%) were symptomatic. Five (12.5%) symptomatic participants in the control group had symptoms of moderate severity (defined as severity score of at least 6) and 18 (45.0%) symptomatic participants in the control group had symptoms of mild severity (defined as severity score of <6). Out of 30 study participants in the control group with confirmed SARS-CoV-2 infection, 19 (47.5%) were symptomatic. The proportions of people with symptomatic SARS-CoV-2 infection and mild symptomatic SARS-CoV-2 infection were lower in the Mito-MES compared to the control group ($p < 0.001$) (Table 2). Among symptomatic SARS-CoV-2 infections, the median duration of symptoms was lower in the Mito-MES group (3.0 days) compared to the control group (5.0 days) (Difference −2.0, 95% CI: −4.0, −1.0) (Table 2). The median days to onset of symptoms was higher in the Mito-MES group (4.5) compared to the control

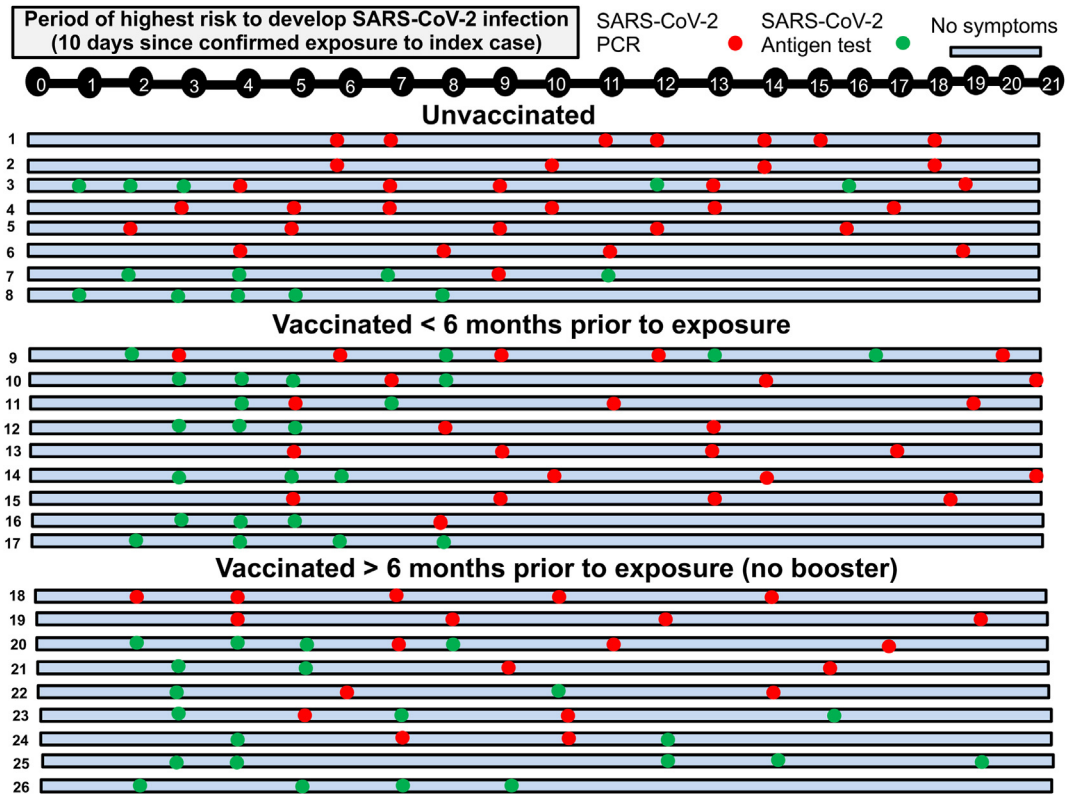

Fig. 3: **Mitoquinone mesylate (Mito-MES) is highly effective post-exposure prophylaxis against development of SARS-CoV-2 infection when taken within 72 h since exposure to index case of confirmed SARS-CoV-2 infection.** Study design as in Figs. 1 and 2. A subgroup of study participants within the Mito-MES group took mito-MES (20 mg daily) for 7 (n = 12) or 14 days (n = 28) within 3 days post exposure (dpe) (n = 40). Twenty-two (55.0%) study participants took Mito-MES within 24 h since high-risk exposure to index case. Four (10.0%) study participants took Mito-MES within 48 h since high-risk exposure to index case. Eight (20.0%), 13 (32.5%) and 19 (47.5%) study participants were unvaccinated or vaccinated within 6 months or vaccinated more than 6 months prior to exposure to the index case, respectively. To be included in the study, all study participants were required to have *at least* 4 SARS-CoV-2 diagnostic tests [PCR in red or rapid antigen test (RAT) in green] if the initial diagnostic tests were negative. The diagram illustrates the use of diagnostic tests with regards to day of exposure (Day 0) and use of Mito-MES within 72 h after exposure.

group (3.0) (Difference 1.5, 95% CI: 0, 2.5) (Table 2). The median severity score of symptoms was also lower in the Mito-MES group (2.0) compared to the control group (3.0) (Difference −1.0, 95% CI: −3.0, −0.5) (Table 2).

The estimates of difference of severity score between the two compared groups was consistent regardless of adjustment for age, sex, smoking status, high risk status and immunization status (Fig. 6). Overall, there was no reliable difference in the severity score in study participants who took Mito-MES for 7 days compared to 14 days regardless of vaccination status, high risk status, smoking, age, and sex (Supplementary Table S4). None of the study participants was hospitalized or required oxygen therapy.

Discussion

This work describes antiviral activity of mito-MES against SARS-CoV-2 in humans. Mito-MES has not previously been tested in humans as an antiviral against acute viral infections. Mito-MES was safe and highly effective at preventing SARS-CoV-2 infection when given within 72 h post high-grade unmasked exposures to SARS-CoV-2. Compared to the control group of study participants who did not take Mito-MES, Mito-MES reduced severity of SARS-CoV-2 infection when given 3–5 days post exposure in study participants after high-risk exposure to SARS-CoV-2. Mito-MES is the only mitochondrial antioxidant approved for oral human use with proven safety in clinical trials for redox stress-related diseases such as Hepatitis C and Parkinson disease.[7] Given that Mito-MES is an over-the-counter safe diet supplement that can be immediately available in humans in the setting of high-grade exposure to SARS-CoV-2, our real-world proof-of-concept open label exploratory clinical trial, suggests that Mito-MES may represent a rapidly applicable post exposure prophylaxis treatment against development of severe SARS-CoV-2 infection.

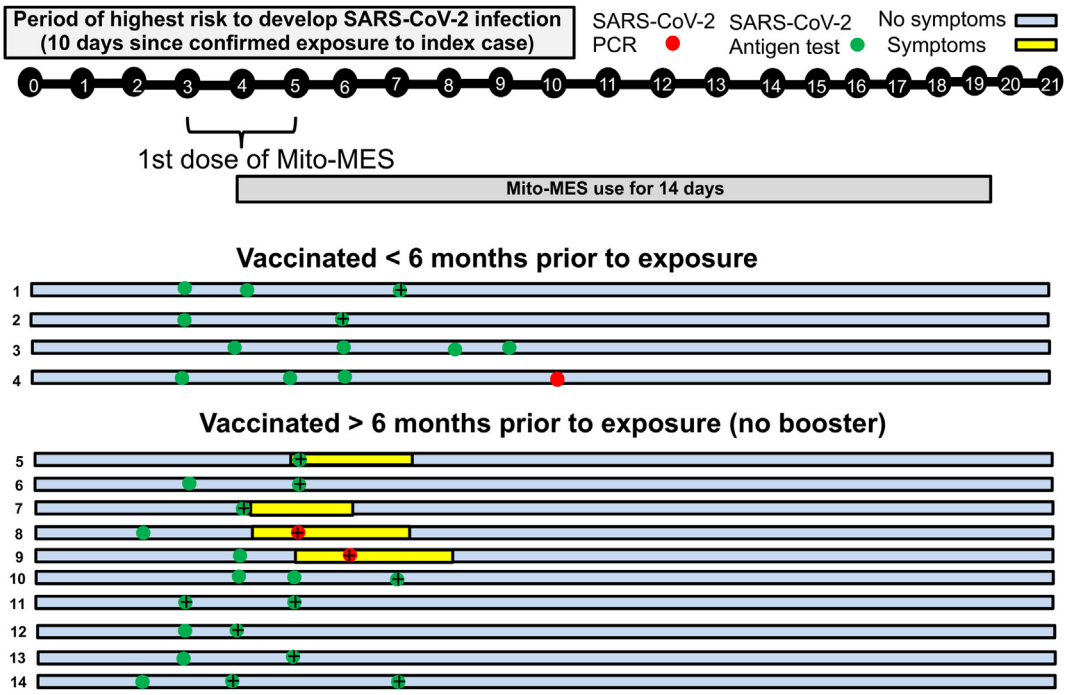

Fig. 4: **Mitoquinone mesylate (Mito-MES) is less effective post-exposure prophylaxis against development of SARS-CoV-2 infection when taken after 72 h since exposure to index case of confirmed SARS-CoV-2 infection.** Study design as in Figs. 1 and 2. A subgroup of study participants within the Mito-MES group took mito-MES (20 mg daily) for 7 (n = 3) or 14 days (n = 11) within 3–5 days post exposure (dpe) (n = 14). 13 (32.5%) and 19 (47.5%) study participants were vaccinated within or more than 6 months prior to exposure to the index case, respectively. To be included in the study, all study participants were required to have *at least* 4 SARS-CoV-2 diagnostic tests [PCR in red or rapid antigen test (RAT) in green] if the initial diagnostic tests were negative. The diagram illustrates the use of diagnostic tests and duration of symptoms of viral illness with regards to day of exposure (Day 0) and use of Mito-MES within 3–5 days after exposure.

We considered the open label design (that has known limitations) as *feasible* and an important *first* step to obtain *proof-of-concept* data that Mito-MES can inhibit development of SARS-CoV-2 infection when given *as soon as possible*. MES was provided to all study participants immediately after informed consent so that it would be available for *immediate* use after *anticipated* exposure to confirmed case of SARS-CoV-2. This study design cannot be *easily* performed in the setting of a large randomized controlled trial (RCT). For example, in the Phase 2/3 EPIC-PEP (Evaluation of Protease Inhibition for COVID-19 in Post-Exposure Prophylaxis) RCT with *unvaccinated* study participants, paxlovid was given within *5 days* post exposure to SARS-CoV-2 (when SARS-CoV-2 is often established) and *failed* to demonstrate therapeutic efficacy (unpublished data). Pragmatic trials offer the ability to produce results that can be generalized and applied in routine practice settings for further testing in definitive large, randomized control clinical trials.[23]

Preclinical experimental studies have shown that blood levels of Mito-MES *do not predict tissue levels* which can be *much higher* than plasma levels due to rapid distribution to tissues.[7,13,15–17] Like other antivirals for SARS-CoV-2 including nirmatrelvir/ritonavir and monoclonal antibodies, pharmacokinetics of Mito-MES have not been studied at the level of not easily accessible human tissues. However, *independent* animal[7,13] and human[8,24] studies (other than our study) have shown potent antiviral[13] or anti-inflammatory activity[8,13,16,24] of Mito-MES and/or its component (CoQ10)[24] in epithelial tissues, confirming that Mito-MES has reliable bioavailability in epithelial tissues to achieve concentrations at the nM level (especially inside the cells). Pharmacokinetics of Mito-MES have not been studied at the tissue level in humans and this has also not been done for other antivirals for SARS-CoV-2 including nirmatrelvir/ritonavir and monoclonal antibodies. Human tissues are not easily accessible for measurement of drug levels of the tissue level. Pharmacokinetics of the much less bioavailable CoQ10 have also not been studied at the tissue level.[25] However, oral administration of CoQ10 in eight patients with COPD at 90 mg/day for 8 weeks improved oxygenation,[24] suggesting adequate penetration in the lung tissue. This dose is several magnitudes higher than the dose use in the current study (20 mg/day) since the *in vivo* bioavailability of Mito-MES is several magnitudes higher than CoQ10.[7,13,15–17] We have also shown that the

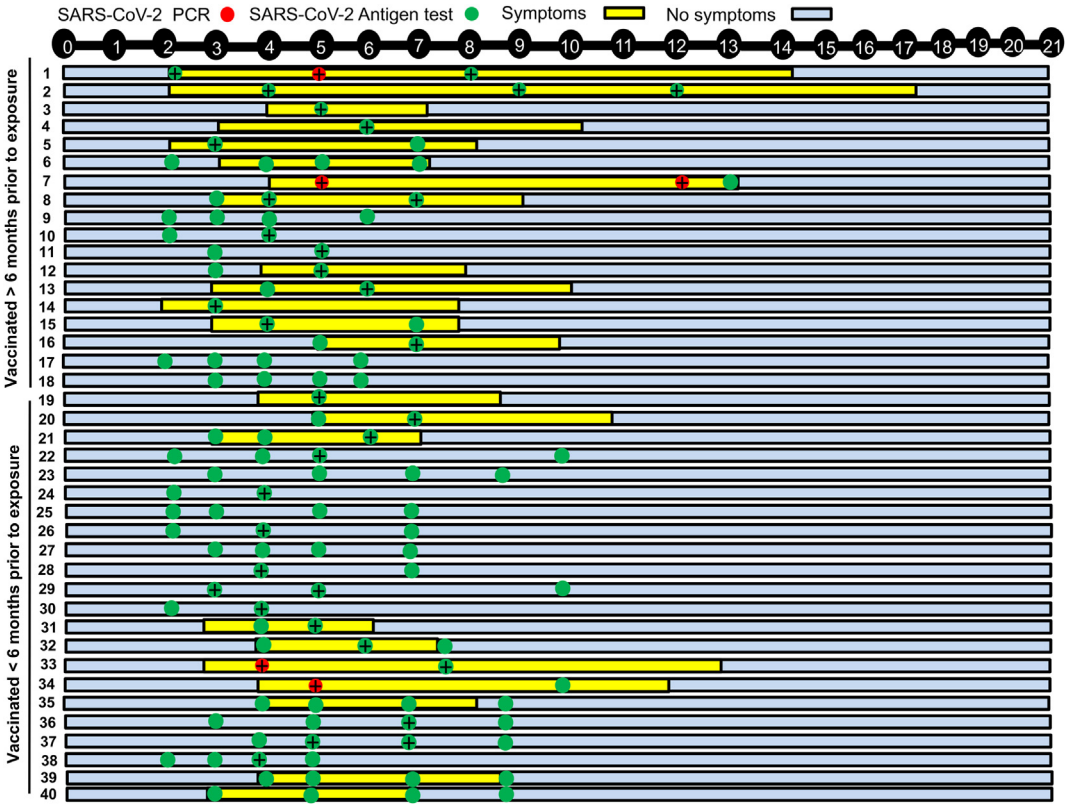

Fig. 5: **Incidence and duration of SARS-CoV-2 infection in the control group who did not take Mitoquinone mesylate (Mito-MES) within 5 days since exposure to index case of confirmed SARS-CoV-2 infection.** Study design as in Figs. 1 and 2. Group B included 40 people who did not take Mito-MES after high-risk exposure to SARS-CoV-2. 18 (45.0%) and 22 (55.0%) study participants were vaccinated after or within 6 months prior to exposure to the index case, respectively. To be included in the study, all study participants were required to have *at least* 4 SARS-CoV-2 diagnostic tests [PCR in red or rapid antigen test (RAT) in green] if the initial diagnostic tests were negative. Thus, group B tended to include people with symptomatic SARS-CoV-2 infection (asymptomatic people in real-world setting do not typically do > 4 SARS-CoV-2 diagnostic tests). Twenty three (57.5%) study participants had symptomatic viral illness while the incidence of symptomatic SARS-CoV-2 in real-world setting is much lower. The diagram illustrates the use of diagnostic tests and duration of symptoms of viral illness with regards to day of exposure (Day 0) and use of Mito-MES within 3–5 days after exposure.

	Mito-MES (N = 40)	Control (N = 40)	Difference (95% CI)	p value
[a]Positive SARS-CoV-2 test, % *(N)*	30.0% (12)	75.0% (30)	−45.0% (−64.5%, −25.5%)	<0.001
[b,d]Days to first positive, *Median (IQR)*	5.0 (4.0, 6.0)	5.0 (4.0, 5.0)	0 (−1.5, 0)	0.400
[a]Presence of symptoms of viral illness, % *(N)*	10.0% (4)	57.5% (23)	−47.5% (−65.4%, −29.6%)	<0.001
[a]Presence of mild viral disease, % *(N)*	10.0% (4)	45.0% (18)	−35.0% (−53.0%, −17.0%)	0.001
[c]Presence of moderate viral disease, % *(N)*	0% (0)	12.5% (5)	0 (−1.0, 0)	0.050
[c]Need for hospitalization, % *(N)*	0% (0)	0% (0)	–	0.999
[b,e]Days to onset symptoms, *Median (IQR)*	4.5 (4.0, 5.25)	3.0 (3.0, 4.0)	1.5 (0, 2.5)	0.02
[b,e]Duration of viral symptoms, *Median (IQR)*	3.0 (2.75, 3.25)	5.0 (4.0, 7.0)	−2.0 (−4.0, −1.0)	0.008
[b,e]Severity score, *Median (IQR)*	2.0 (1.75, 2.25)	3.0 (2.5, 4.5)	−1.0 (−3.0, −0.5)	0.06

Statistical comparison was done between the control and each shown experimental group by using. [a]Two sample proportion test. [b]Two-tailed Mann–Whitney U test and Bootstrap difference in medians. [c]Fisher's exact test. [d]Subset of data of individuals with positive PCR test, Mito-MES group N = 12 and Control group N = 30. [e]Subset of data of individuals with viral symptoms, Mito-MES group N = 4 and Control group N = 23.

Table 2: **Descriptive statistics of participants' measures among compared groups (Mito-MES versus control).**

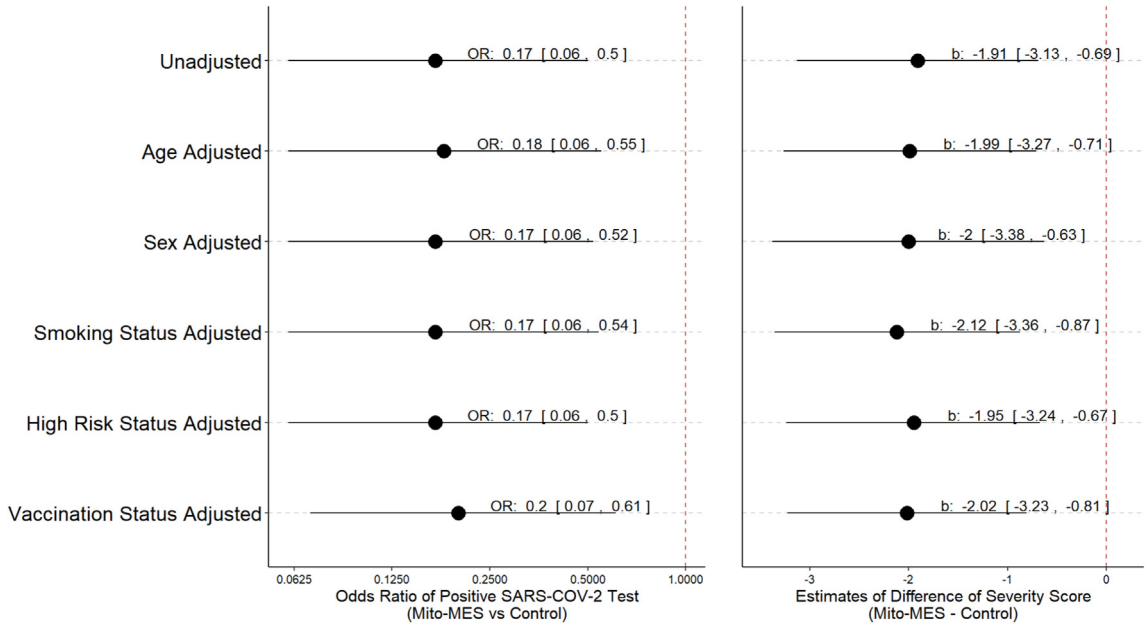

Fig. 6: **Mitoquinone mesylate (Mito-MES) reduces the probability of a positive SARS-CoV-2 diagnostic test and the severity of symptoms after high risk exposure to index case of confirmed SARS-CoV-2 infection.** Study design as in Figs. 1 and 2. Left: Forest diagram that illustrates the odds ratio of a positive SARS-CoV-2 diagnostic test during the study period (14 days) in the Mito-MES compared to the control group. Right: Forest diagram that illustrates the estimates of difference of severity score between the two compared groups (Mito-MES versus control).

antiviral activity of Mito-MES was also mediated through interferon responses.[6] In a randomized, placebo-controlled (RCT) study in healthy volunteers, intranasal interferon given before and after virus challenge with a respiratory coronavirus reduced incidence of colds, the severity of symptoms, and coronavirus replication compared to placebo.[26] Early interferon responses are critical for protection from severe coronavirus disease.[27] Thus, this evidence in combination with our data suggest that a potent anti-SARS-CoV-2 activity of Mito-MES in interferon competent human upper airway cells can potentially protect against SARS-CoV-2 infection.

Mito-MES was safe in our study which is consistent with low incidence rate of side effects with use of 20 mg daily of Mito-MES in prior clinical trials.[7,10] The most common side effects of Mito-MES are nausea and headaches that were dose dependent and have been reported more commonly with doses 40 or 80 mg orally daily in prior clinical trials.[7–9,11,12] No other toxicity was seen.[7–10] Mito-MES has been dispensed as a supplement (10 mg daily) to hundreds of thousand users worldwide. The safety of Mito-MES in humans has been studied in 1 phase I trial of 64 participants[28] and in 5 phase II clinical trials of 222 participants[7–9,11,12] with up to one year follow up[9] as a safe oral agent that has favorable impact on ageing, Hepatitis C and vascular dysfunction but not in Parkinson disease.[7–10] Notably, untargeted versions of mitoquinone; (e.g., CoQ10), are widely used as neutraceuticals.

Our study has limitations. Our clinical trial was not placebo controlled, was open label and was susceptible to selection bias. Since data were self-reported by phone there is inherent reporting bias. Our clinical trial was a small proof of concept *exploratory* study. Although, sample size calculations were not performed in the setting of an *exploratory* study, our study was able to show therapeutic efficacy with regards to the primary endpoint. Since the performance of the EPIC-PEP RCT, rapidly emerging SARS-CoV-2 variants, recurrent SARS-CoV-2 infections within the same person and variable immunizations among people, would necessitate a much larger PEP study in thousands of *vaccinated and/or previously infected people* to demonstrate therapeutic efficacy (prevention of severe disease). This study would not be feasible at this time. Alternatively, our *feasible* small open label study with initiation of intervention *as soon as possible*, showed therapeutic efficacy (prevention of SARS-CoV-2 infection *per se*).

People in the control group in our study did not receive Mito-MES and tended to be symptomatic (57.5%, a higher rate of symptomatic disease than control groups in other COVID-19 studies). This is because asymptomatic people do not often test repeatedly to meet our stringent inclusion criteria with 4 mandatory SARS-CoV-2 negative diagnostic tests (to reliable rule out false negative results). The therapeutic efficacy of Mito-MES outside of the 72-h window for

PEP remains unclear since our small study only included 6 participants who received Mito-MES after 72 h and who had reduced symptom severity and duration of SARS-CoV-2 infection. Compliance was only assessed by questionnaires and not by blood levels of Mito-MES. As outlined in the study design, our study was a real-world pragmatic study of outpatients who by definition were not hospitalized and had mild SARS-CoV-2 infection. The focus of the study was on the exposed household study participants and not the index cases. Thus, the severity score of symptoms in the index cases was not recorded. The exact time of infection in which the index case was positive for SARS-CoV-2 could not be determined in the setting of real-world setting. Our study also has confounding and data from our study populations may not be generalizable to other populations who take post exposure prophylaxis against SARS-CoV-2 infection. Finally, we did not perform quantification of SARS-CoV-2 viral load in nasopharyngeal swabs by PCR and determination of SARS-CoV-2 variants since this was a real-world study outside of the setting of standardized collection of NP swabs and use of identical PCR method to measure SARS-CoV-2. However, the dominant circulating strain in the US at the time of the study was the Omicron (BA.1) SARS-CoV-2 variant.

Our study was pragmatic and may have under-reported harm. Although no major adverse effects were reported by patients using established questionnaires of patient reported outcomes, the collection of safety data was not performed in the setting of a RCT to accurately report side effects. Thus, the incidence of side effects associated with the intervention (Mito-MES) may be higher than reported herein. This limitation of the current study will be addressed by our ongoing RCT (ClinicalTrials.gov identifier NCT05886816).

Generalizability of trial findings is limited to SARS-CoV-2 variants that were studied at the time of this study. Emerging SARS-CoV-2 variants may have higher transmissibility and Mito-MES may not fully inhibit SARS-CoV-2 transmission within 72 h after exposure to emerging SARS-CoV-2 variants. Our findings may not be generalizable in high-risk immunocompromised patients who are at risk for severe SARS-CoV-2 infection.

Thus, despite these limitations, our study succeeded to show highly potent antiviral efficacy with regards to the primary endpoint (development of SARS-CoV-2 infection) *even in unvaccinated cancer patients* (Table 1, Fig. 3). Our human study in combination with our *in vitro* and mouse studies strongly validated the therapeutic efficacy of Mito-MES in SARS-CoV-2 infection.[6] Our study will set the foundation for a needed large RCT of similar design (initiation of Mito-MES within 72 h post exposure to SARS-CoV-2).

To date, there is no safe, efficacious oral antiviral that is effective against SARS-CoV-2 variants and can *also* be given *long term* in humans. The favorable antiviral, antioxidant and anti-inflammatory properties of Mito-MES and its excellent safety profile in humans,[7,10] can establish Mito-MES as a novel therapeutic strategy for outpatient treatment of mild to moderate acute COVID-19, for post-exposure prophylaxis against SARS-CoV-2 in high-risk exposures, for post-acute COVID-19 syndrome (PACS)[29] and as preexposure prophylaxis in high risk (un)vaccinated or immunocompromised patients, where the SARS-CoV-2 vaccines may have low efficacy. These data open potential avenues for randomized control clinical trials of Mito-MES as post (ongoing RCT by study team ClinicalTrials.gov identifier NCT05886816) and pre-exposure prophylaxis against SARS-CoV-2 infection, antiviral treatment of COVID-19 and long-COVID.

Contributors

Conceptualization: TK.
Methodology: NJ, KC, TK.
Investigation: NJ, KC, TK.
Visualization: NJ, KC, TK.
Funding acquisition: TK.
Project administration: TK.
Supervision: TK.
Writing—original draft: TK.
Writing—review & editing: NJ, KC, TK.
All authors read and approved the final version of the manuscript.
All authors (KC, NJ, TK) verified the underlying data.

Data sharing statement

Data are available in the main text or the Supplementary Materials. Anonymized patient data are available from the corresponding author upon reasonable request.

Declaration of interests

This manuscript is related to patents PCT/US2021/040869, US. Application No 63/166,207.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105042.

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