Review Article

Endothelial pulsatile shear stress is a backstop for COVID-19

Marvin A. Sackner[1] and Jose A. Adams[2]

[1]Emeritus Director of Medical Services, Mt Sinai Medical Center of Greater Miami, Miami Beach, FL, U.S.A.; [2]Division Neonatology, Mt Sinai Medical Center of Greater Miami, Miami Beach, FL, U.S.A.

Correspondence: Jose A.Adams (tony.adams@msmc.com)

There has not been any means to inhibit replication of the SARS-CoV-2 virus responsible for the rapid, deadly spread of the COVID-19 pandemic and an effective, safe, tested across diverse populations vaccine still requires extensive investigation. This review deals with the repurpose of a wellness technology initially fabricated for combating physical inactivity by increasing muscular activity. Its action increases pulsatile shear stress (PSS) to the endothelium such that the bioavailability of nitric oxide (NO) and other mediators are increased throughout the body. *In vitro* evidence indicates that NO inhibits SARS-CoV-2 virus replication but there are no publications of NO delivery to the virus *in vivo*. It will be shown that increased PSS has potential *in vivo* to exert anti-viral properties of NO as well as to benefit endothelial manifestations of COVID-19 thereby serving as a safe and effective backstop.

Introduction

The pandemic of COVID-19 produced by SARS-CoV-2 virus is ravaging the world's population and without effective pharmacological treatment or vaccination is expected to confront humanity for years to come. This review deals with the repurpose of a wellness technology initially developed for combating physical inactivity which now targets SARS-CoV-2 virus and its manifestations as COVID-19. By increasing pulsatile shear stress (PSS) to the endothelium, the bioavailability of nitric oxide (NO) and other mediators are increased to provide treatment of complications of COVID-19 and potential inhibition of SARS-CoV-2 replication. Since development, testing safety and demonstrating the effectiveness of a vaccine across diverse populations still requires extensive investigation [1], clinical application of PSS might serve as a backstop for the management of COVID-19.

Anti-viral properties of NO have been demonstrated in human and animal RNA and DNA viruses utilizing *in vitro* exposure to NO donor drugs such as SNAP [2–4]. This drug inhibits the replication of SARS-CoV-2 *in vitro* but has not been utilized *in vivo* [5,6]. Inhalation of NO acts as a vasodilator of the pulmonary vasculature but its rapid reaction with hemoglobin in the pulmonary circulation minimizes its systemic delivery [7].

PSS has been accomplished with motorized platforms that apply reciprocating motion to the body such that pulses formed from changes of fluid inertia are added to the circulation [8,9]. This technology called either pGz or whole-body periodic acceleration (WBPA) up-regulates endothelial nitric oxide synthase (eNOS) that acts upon the amino acid, L-Arginine, to increase NO bioavailability. Mechanotransduction of PSS in the production of NO has recently been investigated by Iring [10] and Roux [11]. eNOS is one of three nitric oxide synthase (NOS) isoforms that act as catalysts to increase NO, the other two being neuronal nitric oxide synthase (nNOS) and inducible nitric oxide synthase (iNOS). eNOS that is present throughout the vascular endothelium increases NO in small quantities as reflected in nMol/l amounts while beneficial to health [12]. nNOS present in neurons and heart muscle increases NO in small quantities and contributes to synaptic plasticity and blood

pressure regulation [13]. The activity of iNOS is unaffected by PSS and it releases large quantities of tissue destructive NO estimated in μMol/l from phagocytic cells as a non-specific immune defense while also serving as an inflammatory mediator and participant in septic shock [13].

WBPA has also been found to increase serum nitrite, prostacyclin, tissue plasminogen activator (tPA), prostaglandin E_2 (PGE2) and adrenomedullin without affecting prothrombin time, activated thromboplastin time, fibrinogen, thrombin time, factor VII and factor VIII [14,15]. PSS can be self- or guided administered using the 'Passive Simulated Jogging Device' (JD), a portable technology based upon its predicate, the motorized platform, that adds pulses to the circulation by motorized foot pedals tapping against a bumper [16].

Other technologies such as external counterpulsation (EECP) delivered by a device that produces PSS has mainly been employed to treat angina pectoris and heart failure. EECP consists of pneumatic cuffs placed on legs and lower torso synchronized for cyclic inflations and deflations that are controlled by the patient's electrocardiogram such that cuffs inflate at the beginning of diastole and deflate at the beginning of systole. This action doubles the number of pulses in the circulation thereby producing PSS. Increased release of plasma nitrite and nitrate into the circulation occurs with this technology and glycemic control in type 2 diabetes is improved [17–20].

We will describe specific features of COVID-19 for which PSS may be of therapeutic value, and draw insights from available experimental data which supports the use of PSS as a novel therapeutic adjunct. Figure 1,
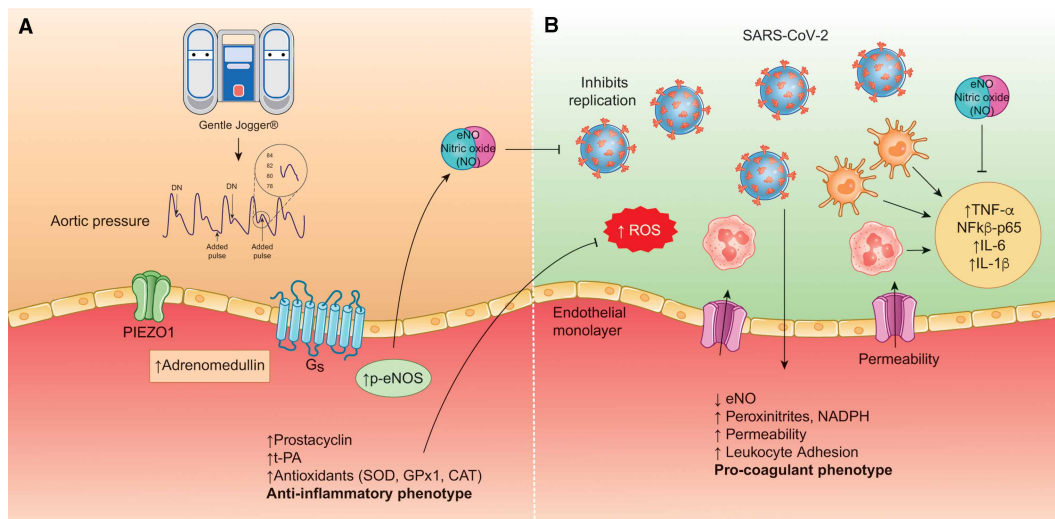

Figure 1. A model of pulsatile shear stress (PSS) effects on a normal (**A**) and SARS-CoV-2-activated (**B**) endothelial monolayer. The left side of the diagram (**A**) depicts a normal endothelial cell monolayer. Gentle Jogger (Jogging Device) induces added pulses to the normal circulation [9]. The dichrotic notch (DN) for each aortic pulse waveform is shown along with the added pulsations induced by Gentle Jogger. Pulsations derived from the normal circulation and those produced by the Gentle Jogger, produce PSS on the vascular endothelium monolayer which activates the cation channel PIEZO1. The latter increases production of adrenomedullin, which via an intermediary step (activates the heterotrimeric G protein (G_s) receptor, leading to activation of protein kinase A(PKA) which activates eNOS by phosphorylation, thus increasing endothelial-derived nitric xide (eNO) [10]. PSS, increases prostacyclin, tissue plasminogen activator (tPA), antioxidants (superoxide dismutase (SOD), glutathione peroxidase 1 (GPx1), catalase (CAT)) and produces an anti-inflammatory endothelium phenotype. The right side (**B**) depicts an activated endothelium from SARS-CoV-2, in which the endothelium monolayer loses its barrier function with increased permeability, reactive oxygen species (ROS) peroxinitrites and NADPH (reduced nicotinamide adenine dinucleotide phosphate) are produced, and the endothelial cell manifests a pro-coagulant phenotype. Bioavailability of nitric oxide is decreased. Additionally, neutrophils and macrophages are stimulated by the virus to produce an increase in the following cytokines; tumor necrosis alpha (TNF-α), nuclear translocation of the NF-kβ-p-65 (nuclear factor kappa beta), and interleukin 6 (IL-6), interleukin 1 beta (IL-1β) and ROS. eNO produced by PSS, inhibits replication of the virus and decreases the production of cytokines. PSS is a means to widely distribute beneficial endothelial derived mediators.

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provides a schematic overview of the effects of PSS on a normal endothelial monolayer, and under the activation of SARS-CoV-2 virus in COVID-19.

Effects of PSS on features of COVID-19

In the past, coronavirus infections were considered solely as respiratory illnesses but now SARS, MERS and COVID-19, that are members of the same coronaviral family have been found to produce diverse, potentially lethal consequences of systemic disease. There are no specific treatments for SARS-CoV-2 itself but preclinical and clinical research indicate that PSS potentially prevents or minimizes multiple aspects COVID-19 that include; (1) viral replication [5,6,21], (2) non-cardiogenic pulmonary edema [22,23], (3) endothelial dysfunction [24,25], (4) coagulopathy [26,27], (5) oxidative stress [28], (6) hyperinflammation [29], (7) cytokine storm [30,31], (8) myocardial injury [32,33], (9) type 2 diabetes [34,35] and (10) hyperglycemia in non-diabetics [36]. Owing to its diverse clinical features and finding the virus in the endothelium [25], Libby and Luscher [37] characterized COVID-19 as an endothelial disease. Mediators released into the circulation with PSS delivered with the motorized platform [38], EECP [17] and Passive Simulated Jogging Device benefit the accompanying features of COVID-19 [16].

Cellular entry of SARS-CoV-2 in host cells

Humans are infected by SARS-CoV-2 by inhaling viral containing droplets and to a lesser extent aerosols that deposit on nasal and airway epithelium when other infected humans in proximity sneeze, shout, talk, cough, breathe or sing [39]. The human host factor angiotensin-converting enzyme 2 (ACE2) is the receptor for the spike protein (S) of SARS-CoV-2 which binds to ACE2 on the host epithelial cells. The main host protease that mediates S protein activation on primary target cells and initial viral entry is type II transmembrane serine protease (TMPRSS2) [40,41]. The S glycoprotein with aid of proteases of the host binds to host cell receptors and fuses the viral membrane with the host cell membrane, an essential process for viral entry. The cysteine protease cathepsin in lysosomes is critical for coronaviral entry through endocytosis [42].

The coronavirus utilizes the enzymatic activity of cathepsin L to infect ACE2-expressing cells, and suppression of cathepsin L leads to inhibition of viral entry and host cell infections. Administration of NO donor drugs inhibits cysteine protease activity which cleave precursor polyprotein(s) that lead to the maturation of infectious virions. Therefore, inhibitors of cysteine protease have potential to block viral replication [43]. NO as delivered with PSS falls into this category. Binding of coronavirus to ACE2 results in receptor-mediated internalization and release of viral RNA for the spread of infection [23]. S-nitrosylation inhibits cysteine proteases encoded within the coronavirus itself through reduction in viral RNA production in early steps of viral replication [24–27].

ACE2, the cellular receptor for coronavirus and TMPRSS2 are co-expressed by type II pneumocytes, indicating that SARS-CoV-2 is cleaved by TMPRSS2 in the lungs of infected individuals. Suppression of this process with NO and/or its derivatives inhibits viral spread and pathogenesis by retention of viral recognition with neutralizing antibodies and inactivation of coronavirus S protein for cell–cell and virus–cell fusion [44].

SARS-CoV-2 replication

In early viral replication, NO or its derivatives reduce palmitoylation of nascently expressed spike (S) protein which affects fusion between the S protein and its cognate receptor, ACE2, as well as decreasing viral RNA production. The latter takes place within 3 h post-infection *in vitro*, possibly due to an effect on cysteine proteases encoded within SARS-CoV-2 [5].

Low doses of sodium nitroprusside, a NO donor drug, inhibit the replication of non-coronaviruses *in vitro* and *in vivo* without cellular toxicity [21]. Although comparable data for coronaviruses have not been reported, this finding suggests that PSS should be evaluated in this regard.

COVID-19 non-cardiogenic pulmonary edema

A prominent clinical feature of COVID-19 is the development of the acute respiratory distress syndrome (ARDS) and acute lung injury (ALI) manifested by non-cardiogenic pulmonary edema that progresses to potentially lethal respiratory failure necessitating mechanical ventilation. Mechanical ventilatory strategies for ARDS have been thoroughly reviewed by Matthay et al. [45] and will not be discussed in this paper. Instead, attention will be directed to the effects of PSS on non-cardiogenic pulmonary edema, coagulopathy, pulmonary hypertension, and survival in a lethal mouse model of *E. coli* lipopolysaccharide (LPS) and meconium

aspiration syndrome (MAS) induced ARDS [22]. It does not appear that SARS-CoV-2 produces unique patho-physiologic features to ARDS although spontaneous coagulation in the micro- and macro-pulmonary vasculature in this condition may be more frequent and intense than found in other causes of ARDS.

Fibrinous thrombi in alveolar arterioles were found in 8 of 10 autopsied COVID-19 patients, attributed to dysregulation of the coagulation and fibrinolytic systems [46]. Furthermore, tissue factor exposed on damaged alveolar endothelial cells and on the surface of leukocyte promoted fibrin deposition, while elevated levels of plasminogen activator inhibitor 1 (PAI-1) from lung epithelium and endothelial cells help create a hypofibrinolytic state [47].

To assess how PSS promotes survival in the presence of pulmonary edema accompanying ARDS, mice were pre-treated with WBPA using a motorized platform, a means to deliver PSS, 3 days prior to LPS i.p. and in another group 30 min after LPS i.p., a dose which killed all mice within 48 h. In another group of mice, L-NAME was provided in drinking water to determine the effect of NO inhibition on survival. All non-treated mice died within 30 h after LPS administration. However, when WPBA was applied 1hr daily for 3 days followed by a single injection of LPS, 60% of mice survived 48 h, the preselected duration of the protocol. In another group of mice, LPS injection followed by 1 h WBPA 30 min later produced 80% survival at 48 h. In these mice, survival persisted beyond 48 h without additional administration of WBPA [33]. All surviving mice appeared normal 14 days after completion of the study and their weight gain, feeding and grooming habits did not differ from non-treated mice. N-Nitroarginine methyl ester (L-NAME), a nonselective NO inhibitor significantly reduced survival which did not exceed 16 h for both non-treated and WBPA treated mice. Therefore, PSS which increases NO is a major contributor to survival in a mouse model of non-cardiogenic pulmonary edema [22].

The microvascular leak is a prominent finding in COVID-19 Infection and lung injury. LPS injected into mice markedly increases microvascular leak in lungs, liver and mesenteric vascular beds. Pre and post treatments with PSS as produced with WBPA reduced microvascular leakage by 50%. LPS administration did not affect angiopoietin tyrosine kinase receptor (TIE2) levels, but significantly reduced phosphorylation of the receptor (p-TIE2), an important receptor involved in inhibiting cellular permeability. Both pre- and post-treatment with WBPA increased TIE2 values by 50%, and restored p-TIE2 to pre LPS values [22].

Pulmonary hypertension is another salient finding in COVID-19 lung injury. Using a model of MAS in swine, which is associated with severe hypoxemia due to intrapulmonary shunt and pulmonary hypertension and serves as a model of ARDS, two groups of anesthetized, paralyzed piglets were studied. One group was maintained on conventional mechanical ventilation (CMV), the other on WBPA. Meconium solution (6 mg/kg) instilled into the trachea caused an immediate rise in mean pulmonary arterial pressure (PAP) from baseline of 15 mmHg to 31 mmHg in CMV piglets and from baseline of 11 mmHg to 26 mmHg in WBPA group. PAP every 15 min over the next 150 min ranged from 21 to 25 mmHg for CMV and 7 to 8 mmHg for WBPA which reduces pulmonary hypertension through the action of NO [48,49].

COVID-19 endothelial dysfunction

In addition to respiratory involvement, major clinical manifestations of COVID-19 such as hypertension, thrombosis of both the macro- and microvasculature, kidney disease, pulmonary embolism, cerebrovascular and neurologic disorders indicate that SARS-CoV-2 targets the endothelium and thus COVID-19 should be viewed in the framework of an endothelial disease. The hallmark of endothelial dysfunction is the suppression of eNOS with concomitant NO deficiency [50,51]. Return toward both normal endothelial function and bioavailability of NO can be accomplished with the application of PSS [52–54].

COVID-19 coagulopathy

The coagulopathy present in COVID-19 is predominantly prothrombotic disseminated intravascular coagulation (DIC) with high venous thromboembolism rates, elevated D-dimer levels, high fibrinogen levels in association with low anti-thrombin levels as well as microvascular thrombosis often in the presence of pulmonary edema. In addition to the high prevalence of central line thrombosis and vascular occlusive events such as ischemic limbs and strokes in critically ill COVID-19 patients, fibrinolytic therapy with tPA in ALI and ARDS improves survival due to the clearing of fibrin deposition from the pulmonary microvasculature and alveoli [55,56].

Approximately 75% of patients who die of COVID-19 meet the criteria for DIC, which is almost exclusively prothrombotic in COVID-19 patients with a thromboembolic complication rate in COVID-19 ICU patients of 31%. In contrast, only 0.6% of patients who survive meet such criteria. Laboratory markers of COVID-19

critical illness include highly elevated fibrinogen levels together with elevated levels of D-dimer. Although COVID-19 pathology reports cite diffuse pulmonary and systemic microvascular thrombosis and occlusion, findings which appear more marked in COVID-19, these occur in ARDS regardless of the cause [55].

Most experts currently recommend prophylaxis of COVID-19 patients with low molecular weight heparin (LMWH) which inhibits the onset of coagulopathy but does not degrade pre-existing deposits of fibrin within the pulmonary microcirculation. PSS from the operation of WBPA safely increases plasma tPA about 2.9-fold over baseline in swine over a 3 h observation period, levels reached within the human therapeutic range of recombinant tPA administration [14]. Utilization of JD daily in humans as part of a wellness strategy should be translational to increase tissue-type plasminogen activator (tPA) in the low therapeutic range of fibrinolytic therapy but its effectiveness needs confirmation in human clinical trials.

COVID-19 oxidative stress

Increased oxidative stress produced during ALI/ARDS is a target not yet well investigated. However, PSS administered through WBPA up-regulates expression of endogenous antioxidants from the endothelium that include glutathioneperoxidase-1 (GPX-1), catalase (CAT), superoxide dismutase 1 (SOD1) and nuclear factor erythroid related factor-2 (NRF2). These substances ameliorate oxidative stress which often accompanies inflammatory states [57].

COVID-19 hyperinflammation

Acute progression of COVID-19 consists of three phases: (1) an early infection phase, (2) a pulmonary phase and (3) a severe hyperinflammation phase. These phases may overlap in any given patient. During the early infection phase, the virus infiltrates the pulmonary parenchyma and proliferates. In this stage, there are mild constitutional symptoms and the initial response by innate immunity is outpouring of monocytes and macrophages. Collateral tissue injury and the inflammatory processes that follow in phase 2 such as vasodilation, endothelial permeability and leukocyte recruitment lead to further pulmonary damage, hypoxemia and cardiovascular stress. In a small fraction of patients, the host inflammatory response intensifies even with diminishing viral loads and produces systemic inflammation with the potential to injure distant organs. The development of myocarditis without evidence of direct viral infiltration implicates the heart as one such target. This represents an advanced stage of the acute hyperinflammatory phase characterized by multiple organ failure and elevation of key inflammatory markers that include among others interleukins (IL-6, IL-2, IL-7), and tumor necrosis factor alpha (TNF-α). Daily application of JD that produces PSS as prophylaxis and treatment during any of the three phases benefits health by suppressing inflammatory cytokines [58].

COVID-19 cytokine storm

Another clinically important feature of SARS-CoV-2 infection is cytokine storm that can occur late in the course of ARDS found in COVID-19. It is marked by elevated levels of TNF-α, nuclear factor kappa beta (NF-κβ-p65), IL-1β and IL-6. Preclinical prevention of this often lethal condition with PSS delivered by WPBA reduces levels of these inflammatory cytokines and increases the anti-inflammatory cytokine IL-10 four-fold above its baseline values [58]. There has been no effective clinical means to prevent and minimize cytokine storm and PSS delivered with JD deserves a human trial.

COVID-19 myocardial injury

From 20% to 36% of hospitalized patients with COVID-19 develop acute myocardial injury characterized by elevation of high-sensitivity troponin and/or heart muscle fraction of creatine kinase isoenzyme (CK-MB). This clinical entity has been attributed to one or more of the following factors: (1) hyperinflammation and cytokine storm mediated through pathologic T-cells and monocytes leading to myocarditis, (2) respiratory failure and hypoxemia damaging cardiac myocytes, (3) down-regulation of ACE2 expression and subsequent protective signaling pathways in cardiomyocytes, (4) hypercoagulability and coronary microvascular thrombosis, (5) endothelial dysfunction and (6) inflammation and/or stress causing coronary plaque rupture or supply demand mismatch leading to myocardial ischemia/infarction. Such patients may be asymptomatic or develop fulminant myocarditis and circulatory shock [33,59].

There have been preclinical and clinical studies of PSS administered with WBPA or JD that provided benefits to cardiac health which might mitigate myocardial injury in COVID-19. These include (1) increased microcirculatory blood flow to the epicardium and endocardium of anesthetized swine [60], (2) improvement of coronary

flow reserve (CFR) in healthy humans, patients with coronary artery disease, and type 2 diabetic subjects [61–63], (3) improvement of exercise capacity, myocardial ischemia and left ventricular (LV) function over 4 weeks daily trial of PSS in patients with angina [64], and increase in heart rate variability in normal subjects [65].

COVID-19 type 2 diabetes and hyperglycemia in non-diabetics

COVID-19 patients without other comorbidities but with rype 2 diabetes are at higher risk of ARDS, excessive uncontrolled inflammatory responses, hypercoagulable state and more susceptible to cytokine storm that may lead to rapid deterioration of clinical state in COVID-19 [35]. Diabetic patients with COVID-19 have increased disease severity and a higher risk of mortality [66]. Elevation of the initial blood glucose level of hospitalized non-diabetic patients with COVID-19 is an independent risk factor for in-hospital mortality [66]. Therefore, tight glycemic control of blood glucose variability in both diabetic and non-diabetic patients with COVID-19 should be a goal of therapy.

Blood glucose-lowering agents such as metformin, sulfonylurea derivatives and insulin all can improve glycemic control in type 2 diabetics, but these agents have limited or no effect on hypertension often associated with diabetes present in COVID-19 [67]. Both hypertension and glycemic variability can be controlled with JD during the same treatment sessions [16,67,68].

In 11 type 2 diabetics and 11 healthy subjects, self-administered JD a minimum of 3 times per day for 30 min per day for 7 days at home was assessed with continuous glucose monitoring. In both diabetic and healthy subjects, 24 h blood glucose values were reduced. This effect was most likely related to NO released by PSS stimulates glucose transport in skeletal muscles by increasing glucose transporter type 4 (GLUT-4) levels at the cell surface [69].

Conclusion

Endothelial PSS is a means to widely distribute beneficial mediators to health such as nitric oxide, prostacyclin and tPA among others throughout the body. Although there has not yet been an effective treatment for COVID-19, PSS offers non-invasive and safe means to provide treatment and prevention of aspects of this pandemic.

Summary

- PSS is a novel preventative and therapeutic modality to combat COVID-19

- PSS enhances the production of mediators which are important in various physiological derangement of COVID-19, such as cytokine storm, coagulopathy, myocardial injury and hyperglycemia

- PSS is an adjunct intervention to be used in viral and bacterial illnesses which have similar physiologic derangement as COVID-19

Competing Interests

No funding was received for this review. Drs. Sackner and Adams draw no salary from Sackner Wellness Products LLC. Dr. Sackner owns 80% and Dr. Adams 20% of the domestic and foreign patents.

Author Contribution

M.A.S. and J.A.A. contributed equally to this work.

Abbreviations

ACE2, angiotensin-converting enzyme 2; ALI, acute lung injury; ARDS, acute respiratory distress syndrome; CMV, conventional mechanical ventilation; DIC, disseminated intravascular coagulation; EECP, external counterpulsation; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; LPS,

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lipopolysaccharide; MAS, meconium aspiration syndrome; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; PSS, pulsatile shear stress; PSS, pulsatile shear stress; tPA, tissue plasminogen activator; WBPA, whole-body periodic acceleration.

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