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Letter to the Editor

Blood nitrate and nitrite modulating nitric oxide bioavailability: Potential therapeutic functions in COVID-19

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ABSTRACT

Most outcomes of COVID-19 are associated with dysfunction of the vascular system, particularly in the lung. Inhalation of nitric oxide (NO) gas is currently being investigated as a treatment for patients with moderate to severe COVID-19. In addition to the expected vasodilation effect, it has been also suggested that NO potentially prevents infection by SARS-CoV-2. Since NO is an unstable radical molecule that is easily oxidized by multiple mechanisms in the human body, it is practically difficult to control its concentration at lesions that need NO. Inorganic nitrate and/or nitrite are known as precursors of NO that can be produced through chemical as well enzymatic reduction. It appears that this NO synthase (NOS)-independent mechanism has been overlooked in the current developing of clinical treatments. Here, I suggest the missing link between nitrate and COVID-19 in terms of hypoxic NO generation.

Lung injury caused by coronavirus disease 2019 (COVID-19) often progresses rapidly with acute respiratory distress syndrome (ARDS) followed by multiple organ failure due to a “cytokine storm”. A recent randomized clinical trial has suggested that the use of the corticosteroid dexamethasone lowers mortality among those who were receiving either invasive mechanical ventilation or oxygen alone [1]. To date, however, there are no promising specific drugs or treatments for the severe hospitalized patients. High-dose intravenous vitamin C (HDIVC) treatment has been reported to be effective in decreasing days of hospitalization, ICU and mortality [2]. Vitamin C (Vit C, L-ascorbic acid) therapy has been known for several decades as a safe adjunctive treatment that has been examined in a wide variety of diseases including the severe acute respiratory syndrome (SARS) caused by SARS-CoV [3]. In spite of its long historical background, there yet exist controversies on HDIVC treatment [4]. Here I draw attention to blood nitrate and nitrite that potentially modulate nitric oxide (NO) bioavailability in response to Vit C levels.

SARS-CoV-2 binds to ACE2 receptors on alveolar epithelial type II (ATII) cells in the lung [5]. After the infection, the ATII cells recruit alveolar macrophages (AM) that scavenge the virus along with releasing immune signals such as cytokines [6]. These cells require a high concentration of intercellular Vit C (mM) for sustaining their pivotal functions in innate immunity [7]. Humans obligately ingest this essential vitamin from daily diets due to the lack of L-gulono-γ-lactone oxidase enzymes. Vit C in plasma is incorporated into cells by sodium-dependent Vit C transporters. In parallel, dehydroascorbic acid (DHA), an oxidized form of Vit C, is taken up through glucose transporters. Since glucose competes with DHA on the transporters [8], Vit C availability in the cells may be limited in high blood sugar conditions, a potential reason for pathological severity of COVID-19 for diabetes patients.

Vit C is a potent natural antioxidant that primarily removes reactive oxygen species (ROS) which are over-produced in inflammation. In addition to such antioxidant function, the vitamin plays pleotropic roles in human physiology. It has been known that Vit C is associated with NO production through chemical reaction with inorganic nitrite (NO_2^-) [9,10]. Since the reaction is significant in acidic conditions, its physiological relevance has been considered only in gastric juice of the stomach [11]. In lung injury with respiratory and/or metabolic acidosis such as in ARDS, a low local pH in the damaged capillary might allow for the chemical production of NO from nitrite. Even at a near neutral pH, nitrite can be reduced to NO in hypoxic conditions by multiple enzymes [9,11], such as deoxy-hemoglobin (deoxy-Hb) in erythrocytes, xanthine oxidoreductase (XOR) in the blood during infection, or cytochrome oxidase (COX) of mitochondrial respiratory chain(Fig. 1).

NO is a short-lived gaseous free radical that controls vasodilation, which is expected to reverse pulmonary hypertension in COVID-19 [12]. Recently, investigation of the therapeutic effects of inhaled NO (iNO) on COVID-19 has been proposed [12,13]. It is of interest that there were few asthma patients with severe cases of COVID-19 in China [14]. NO emission in asthmatic patients is high due to T-helper cell type 2 (Th2)-mediated airway inflammation. To assess and manage asthma, the fraction exhaled NO (FeNO) has been adopted as a non-invasive indicator of airway inflammation. In addition to vasodilation, such inhaled and exhaled NO may have substantial antiviral activity against SARS-CoV-2 infection as was suggested for SARS-CoV [12]. Although cigarette smoking has been listed as a risk factor for contracting COVID-19, only a low proportion of the smokers have suffered from SARS-CoV-2 infection in China, Europe and the U.S. The intermittent burst of high NO concentration in cigarette smoke has been proposed as a mechanism in protecting against the virus infection [15].

Cardiovascular diseases such as hypertension have been recognized as the most frequent comorbidities in patients with COVID-19. It is conceivable that lower or impaired NO metabolism is associated with the pathological severity of COVID-19. HDIVC might help to supply NO on demand through its chemical reaction with nitrite. NO is primarily synthesized by NO synthase (NOS) enzymes in humans. It is important to

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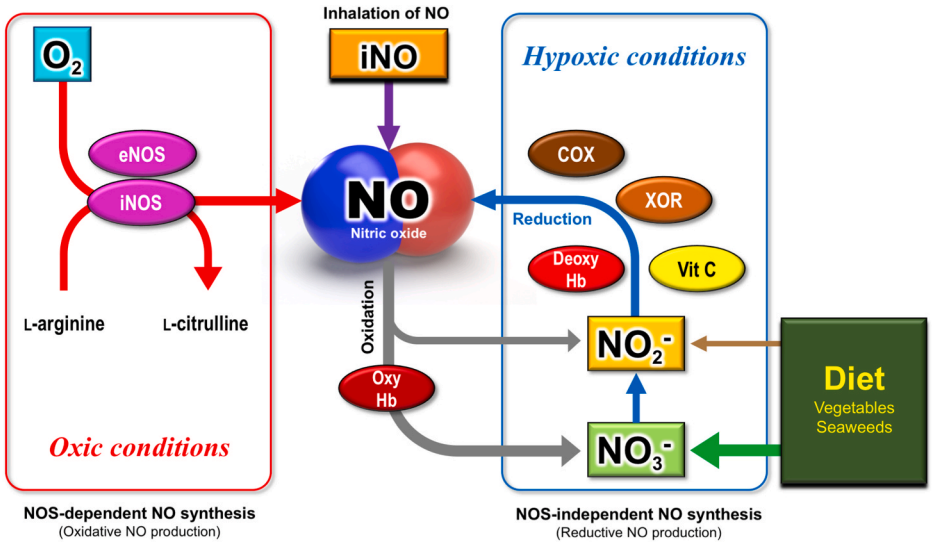

Fig. 1. Schematic illustration for NO generation pathways. Nitric oxide (NO) displays multiple physiological functions in humans such as vasodilation effects. It has been also suggested that NO inhibits the replication of viruses including SARS-CoV, thereby preventing viral infection. There are two distinct mechanisms for NO synthesis, namely, NOS-dependent and NOS-independent NO generating mechanisms. Regardless of the pathways, inorganic nitrate (NO_3^-) and/or nitrite (NO_2^-) are produced as the oxidation product of NO. In humans, NO_3^- is supplied in daily diets including green vegetables or seaweeds, which may help to support the NO bioavailability. NO, nitric oxide; iNO, inhaled nitric oxide, NOS, nitric oxide synthase; eNOS, endothelial NO synthase; iNOS, inducible NO synthase; oxy-Hb, oxy-hemoglobin; deoxy-Hb, deoxy-hemoglobin; XO, xanthine oxidoreductase; COX, cytochrome oxidase: NO_2^-, inorganic nitrite: NO_3^-. Inorganic nitrate; Vit C, vitamin C (L-ascorbic acid).

note that NO synthesis by NOS requires oxygen and is thereby inhibited in ARDS due to hypoxia. As the consequence, the NO oxidation products nitrite and/or nitrate in plasma are also expected to be low. Inconsistencies in the results of previous clinical trials of Vit C therapy may be due, in part, to variation of nitrite and/or nitrate levels in plasma among the patients.

In contrast with Europe and U.S., the capita death rate from COVID-19 appears, so far, low in East Asia including Japan. The Japan paradox has recently received much attention from researchers as well as from the public [16]. In terms of a biological rationale, it can be hypothesized that nitrate-rich dietary foods in those countries may supplement NO bioavailability through the NOS-independent mechanisms. In a good agreement with this prospect, Japanese diet containing abundant nitrate has been reported to improve hypertension and other vascular diseases [17]. A recent randomized clinical trial has also supported the effects of leafy green vegetables rich in nitrate on blood pressure [18]. Until specific drugs are developed for COVID-19, blood nitrate and nitrite that modulate NO bioavailability are worthy of consideration not only for clinical treatment but also for the prevention of SARS-CoV-2 infection.

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