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Severe acute respiratory syndrome coronavirus 2 may cause liver injury *via* Na⁺/H⁺ exchanger

Cumhur Cure M *et al.* SARS-CoV-2 Causes Liver Injury *via* NHE

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Abstract

The liver has many significant functions, such as detoxification, urea cycle, gluconeogenesis, and protein synthesis. Systemic diseases, hypoxia, infections, drugs, and toxins can easily affect the liver, which is extremely sensitive to injury. Systemic infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can cause liver damage. The primary regulator of intracellular pH in the liver is Na⁺/H⁺ exchanger (NHE)₁. Physiologically, NHE protects hepatocytes from apoptosis by making the intracellular pH alkaline. SARS-CoV-2 increases local angiotensin II levels by binding to angiotensin-converting enzyme 2. In severe cases of coronavirus disease 2019, high angiotensin II levels may cause NHE overstimulation and lipid accumulation in the liver. NHE overstimulation can lead to hepatocyte death. NHE overstimulation may trigger a cytokine storm by increasing proinflammatory cytokines in the liver. Since the release of proinflammatory cytokines such as interleukin-6 increases with NHE activation, the virus may indirectly cause an increase in fibrinogen and D-dimer levels. NHE overstimulation may cause thrombotic events and systemic damage by increasing fibrinogen levels and cytokine release. Also, NHE overstimulation causes an increase in the urea cycle and vitamin D synthesis while inhibiting gluconeogenesis in the liver. Increasing NHE₃ activity leads to Na⁺ loading, which impairs the containment

and fluidity of bile acid. NHE overstimulation can change the gut microbiota composition by disrupting the structure and fluidity of bile acid, thus triggering systemic damage. Unlike other tissues, TNF- α and angiotensin II decrease NHE₃ activity in the intestine. Thus, increased luminal Na⁺ leads to diarrhea and cytokine release. SARS-CoV-2-caused local and systemic damage can be improved by preventing the virus-induced NHE overstimulation in the liver.

Key Words: Liver; Hepatocyte; Severe acute respiratory syndrome coronavirus 2; COVID-19; Na⁺/H⁺ exchanger; Sodium-proton pump

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Core Tip: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) readily infects the liver containing angiotensin-converting enzyme 2. Increased angiotensin II causes NHE overstimulation. NHE overstimulation renders the accumulation of Na⁺ and Ca²⁺ in hepatocytes. Thus, hepatocytes get damaged and eventually die. Besides, raised cytokine release increases fibrinogen levels, enhancing thrombotic events. Cytokine storms can be triggered by NHE overstimulation. Also, vitamin D synthesis can be impaired. SARS-CoV-2-induced NHE overstimulation can change bile acid structure. Changed content and fluidity of bile acid may disrupt gut microbiota. Disrupted gut microbiota can also trigger cytokine storms. Liver damage from the virus can be considered the most important cause of disease progression and mortality.

INTRODUCTION

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been causing a worldwide pandemic and multisystem organ involvement, resulting in immense hospital costs and mortality. Although the virus settles in the lungs and causes infection, it spreads to organs, including angiotensin-converting enzyme (ACE) 2, such

as the heart, liver, and kidney, *via* neighborhood route, systemic blood circulation, or vascular endothelium^[1,2]. Liver involvement is also often seen in the course of severe novel coronavirus disease 2019 (COVID-19) and may advance the progression of the disease^[3]. Understanding how SARS-CoV-2 spreads to these organs and solving the damage mechanism in the organs will provide an outstanding opportunity to reduce the severity and mortality of the disease.

Intracellular pH plays a vital role in SARS-CoV-2 infection^[4]. Physiologically, the pH of endosomes is low. Therefore, acidic pH leads to the autophagy of viruses and harmful substances^[4]. However, SARS-COV-2 can smoothly escape autophagy by manipulating cellular autophagy^[5], and causing infection at low intracellular pH by fusing with ACE2^[6]. When the pH of the endosomes becomes alkaline, SARS-CoV-2 can not infect the cell since the configuration of ACE2 changes^[7]. Hydroxychloroquine, which makes the intracellular pH alkaline, has been used until its harmful effects appear in patients with COVID-19^[8]. The primary regulator of intracellular pH in the liver and many organs is Na⁺/H⁺ exchanger (NHE)₁^[9,10]. In a previous study, NHE activity was high in the blood of patients with COVID-19^[11]. NHE activation in COVID-19 has been associated with cytokine storms and organ damage^[12]. Unraveling the unique relationship between NHE and SARS-CoV-2 may illuminate liver involvement, mortality, and progression of COVID-19. Therefore, we should solve the mechanism of possible SARS-CoV-2 and NHE interaction in liver tissue.

COVID-19 AND LIVER

The liver has many significant functions, such as detoxification, urea cycle, gluconeogenesis, and protein synthesis. Many systemic diseases, hypoxia, infections, drugs, and toxins can easily affect the liver, which is extremely sensitive to injury. Systemic infection of SARS-CoV-2 or agents used in the COVID-19 treatment can cause liver damage^[13]. Liver enzyme elevation is seen in 16.1%-53.1% of patients with COVID-19^[14]. Liver damage has been seen in approximately 20% of hospitalized patients with

COVID-19^[14]. Since hepatocytes express ACE2, SARS-CoV-2 can directly cause liver involvement^[15].

In COVID-19, gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) enzymes, which indicate cholestasis, increase as well as aspartate aminotransferase (AST) and alanine transaminase (ALT), which show hepatocyte damage^[16]. AST, ALT, GGT, and bilirubin indicate liver damage but also reflect the severity of COVID-19^[13,17]. Liu *et al*^[16] have revealed that GGT and ACE2 share the same transcriptional machinery and have speculated that GGT may indicate the *in vivo* expression level of ACE2. Besides, the finding of high bilirubin as a predictive marker for mortality in patients with COVID-19 reveals the importance of liver involvement in the disease^[18,19]. On the other hand, the presence of microthrombus in most patients with COVID-19 and the fact that arterial thrombosis is responsible for mortality make liver involvement more significant in COVID-19. Fibrinogen, the precursor of fibrin, is synthesized in the liver. D-dimer, a fibrin degradation product, was elevated in many critically ill COVID-19 patients^[20]. D-dimer levels correlate with total bilirubin, AST, and ALT levels in patients with COVID-19^[21]. Baroiu *et al*^[21] reported that D-dimer might be a predictive marker of abnormal liver function parameters and liver injury in patients with COVID-19. Therefore, in most patients with COVID-19, liver involvement may be responsible for mortality and the disease progression.

NHE AND ITS ISOFORMS

There are many ion pumps in plasma membranes. They provide signaling and stabilization of ion concentrations between intracellular and extracellular areas. NHE is one of the most important of them and has nine isoforms. These isoforms are found in different tissue and cellular localizations and have various functions depending on their localization. NHE₁ is the cleaning form found in almost all tissues and is expressed abundantly in the liver. NHE₂ is located in the stomach and intestines. Liver, intestine, and kidney tissues have NHE₃. NHE₄ is in the stomach and kidney. The brain has

NHE₅. NHE₆₋₉ are found in intracellular organelles^[22-24]. NHEs are involved in the etiology of several gastrointestinal and liver diseases^[22].

NHE AND ITS FUNCTION IN LIVER

NHE is localized in the basolateral or sinusoidal membrane of hepatocytes^[22]. The NHE, which provides the intracellular pH balance, is physiologically activated when the intracellular pH decreases and brings the intracellular pH to its physiological levels^[25]. NHE causes the movement of Na⁺ into the cell and H⁺ out of the cell. As soon as the intracellular pH reaches physiological levels, the NHE activity reduces^[12]. NHE provides the passage of Na⁺ into the cell. The Na⁺/K⁺/2Cl⁻ cotransporters cause the influx of Na⁺, K⁺, and Cl⁻ into the cell. Simultaneously, the Cl⁻/HCO₃⁻ antiporter ensures the influx of Cl⁻ into the cell. Na⁺/K⁺-ATPase (NKA) pumps Na⁺ outward while K⁺ moves into the cell. Eventually, KCl increases in hepatocytes^[25-27]. Na⁺/Ca²⁺ Exchanger (NCE) provides intracellular Ca²⁺ balance^[28]. These pumps work in harmony in the liver. Physiological NHE, NCE, and NKA activities are summarized in Figure 1.

In acute ischemic events, NHE activation increases intracellular pH and prevents hepatocytes from undergoing apoptosis^[10]. Arginine vasopressin activates NHE in hepatocytes by calcium/calmodulin-dependent processes^[29]. Stimulation of NHE is involved in hepatocyte regeneration and growth^[25]. However, if the stimulus is increased, hepatocyte apoptosis due to intracellular Ca²⁺ accumulation increases^[22,25].

Chronic cytokine or platelet-derived growth factor-mediated NHE stimulation causes hepatic stellate cell proliferation and fibrosis^[30]. NHE₃ is located in the apical membranes of the hepatocytes and cholangiocytes. NHE₃ is responsible for maintaining the fluid content of bile acid^[31]. Increased NHE₃ activity leads to Na⁺ loading and an increase in the concentration function of the gallbladder, resulting in gallstone formation^[32-34].

PROLONGED NHE ACTIVATION

While NHE does significant work in physiological conditions, its continuous activation causes serious problems. During NHE physiological function, NCE pumps Na^+ into the cell and Ca^{2+} outward. As a result of NHE overstimulation, NCE activation stops, and reverse NCE becomes active due to increased Na^+ in the cell. While Ca^{2+} is pumped into the cell, Na^+ begins to be pumped outward. As a result, the concentration of Na^+ and Ca^{2+} in the cell increases. The NKA pump, which pumps K^+ inward and Na^+ outward, loses its function due to increased intracellular Na^+ and ATP depletion^[4,12,35-37]. While edema occurs due to Na^+ accumulation in hepatocytes, Ca^{2+} overload causes hepatocyte apoptosis^[38]. In the prolonged activity of NHE, continuous pumping of H^+ into the extracellular area causes some chain redox reactions, and H^+ begins to influx into the cell, and the intracellular pH drops^[4,12,29]. NHE overstimulation and its outcomes are summarized in Figure 1. NHE activation also increases the influx of lipids and free fatty acids (FFAs) into the cell. FFAs influx further increases intracellular acidity, and concomitant Ca^{2+} overload accelerates apoptosis^[36]. As a result of all these events, acute hepatocyte damage may progress from an advanced level to liver failure. Tanaka *et al*^[39] found that NHE suppression inhibited the nuclear factor kappa B (NF- κ B) pathway and proinflammatory cytokine release, thus preventing fatal acute liver failure. Besides, there is a strong interaction between NHE and low-density lipoprotein receptors (LDLR), which may cause liver damage. Non-physiological LDLR excess gives rise to cholesterol overload in hepatocytes. Lipid deposition causes hepatomegaly in the chronic process^[40]. Although hepatocytes are resistant to cholesterol loading, hepatocytes begin to die with prolonged lipid deposition^[40].

SARS-CoV-2 AND NHE INTERACTION

The alkalinity of the intracellular pH creates a barrier against SARS-CoV-2 infection. Therefore, this mechanism has been considered for COVID-19 treatment^[6]. Hydroxychloroquine has been used for COVID-19 treatment since it makes the intracellular pH alkaline by inhibiting Vacuolar H^+ -ATPase (V-ATPase)^[41]. Also, metformin converts endosome pH to alkaline *via* V-ATPase^[42-44]. At or slightly above

the physiological NHE activation can shift the intracellular pH to alkaline, preventing ACE2-SARS-CoV-2 fusion. Using proton pump inhibitors in mild COVID-19 patients may worsen the course of the disease^[45,46]. However, when the virus takes total control of the cells in patients with severe COVID-19, NHE overstimulation causes many detrimental effects, as well as a decrease in intracellular pH.

Hitherto, it has not been determined whether SARS-CoV-2 directly affects NHE; however, NHE activity has been found higher in the blood of patients with COVID-19^[11]. SARS-CoV-2 can increase NHE activity by many mechanisms. SARS-CoV-2-infected mice, which have physiological angiotensin II levels, have been shown to have no damage in some of their tissues^[47]. However, severe patients with COVID-19 have high angiotensin II levels^[48]. ACE2 degrades angiotensin II and reduces its levels. When SARS-CoV-2 fuses with ACE2, the level of angiotensin II increases in the circulation and tissues since ACE2 can not complete its task^[4]. The liver has a local renin-angiotensin system (RAS) system, which is not very important in physiological conditions, dissimilar to hepatic RAS, which plays a significant role in pathological conditions^[49,50]. Since the liver has ACE2, the virus increases the local angiotensin II level by binding to ACE2 in the liver. Angiotensin II is the substantial stimulus of NHE. Angiotensin II shows pro-oxidant, fibrogenic, and pro-inflammatory actions on the liver^[51].

SARS-CoV-2 uses lipid rafts and cholesterol to fuse with ACE2 in membranes^[52]. Lipid rafts play a remarkable role in the entry of SARS-CoV-2 into the cell^[53]. Besides, there is a strong interaction among lipid rafts, cholesterol, and NHE. Therefore, the virus can activate NHE through cholesterol and lipid rafts^[36]. The release of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF- α) and interleukin (IL)-6 and raised oxidative stress by SARS-CoV-2 can also stimulate NHE^[12]. Fibrinogen produced in the liver provides activation of NHE₁ by inducing NCE^[54]. Fibrinogen increase in COVID-19 can also initiate NHE activation.

SARS-CoV-2 AND NHE IN THE LIVER

Since the liver has many vital functions, the virus infecting it will have many systemic consequences. Physiologically, NHE protects hepatocytes from apoptosis by making the intracellular pH alkaline^[10]. However, NHE overstimulation can cause acute lethal liver damage^[39]. Once the virus settles in the liver and infects hepatocytes, it can cause NHE overstimulation. The virus renders intracellular Ca^{2+} accumulation with reverse NCE activation after Na^+ accumulation in hepatocytes. Apoptosis is induced in hepatocytes and cell death. Liver enzymes such as AST and ALT increase due to parenchymal damage^[55]. NHE overstimulation may also trigger a cytokine storm by increasing proinflammatory cytokines in the liver^[12]. On the other hand, NHE_1 plays a role in urea synthesis^[56]. Increased H^+ extrusion increases urea synthesis^[57]. The virus can increase the level of urea by NHE_1 overstimulation. Increased urea in patients with COVID-19 indicates kidney damage^[58]. However, urea may also be a marker of liver injury. Gluconeogenesis in the liver is significantly reduced when the intracellular pH is lowered. Therefore, a hypoxic environment occurs^[59,60]. In physiological conditions, NHE stimulates gluconeogenesis by increasing intracellular pH. NHE overstimulation decreases gluconeogenesis in the liver by reducing intracellular pH^[61]. In severe patients with COVID-19, elevated blood glucose may be due to insulin resistance^[61] or decreased insulin secretion related to NHE and proinflammatory cytokines-mediated pancreatic damage^[62]. Other liver functions are vitamin B_{12} storage^[63], iron storage^[64], and vitamin D synthesis^[65]. Vitamin D reduces acute liver injury^[66]. Vitamin D is immunomodulatory and low levels may worsen the progression of COVID-19^[67,68]. Insulin-like growth factor (IGF)-1 Level^[69,70], which stimulates vitamin D synthesis in hepatocytes, was lower in COVID-19 patients^[71]. While vitamin D increases circulating IGF-1 Levels^[72], IGF-1 inhibits NHE. Increased intracellular Ca^{2+} decreases vitamin D synthesis in the liver^[73]. NHE-mediated hepatocyte injury in patients with COVID-19 may reduce vitamin D synthesis and worsen disease progression. Reduced intracellular pH leads to iron release from the liver^[74], and excess iron causes local and systemic damage with the Fenton reaction^[75]. Low intracellular pH caused by NHE overstimulation can lead to iron-mediated damage to cells in patients with COVID-19.

According to the LDLR mechanism we described earlier, the virus can cause lipid accumulation in the liver, leading to the fattening and enlargement of the liver and even loss of function^[36]. Unfortunately, there is not enough information in the literature regarding the role of LDLR in patients with COVID-19. Although a study proved the opposite^[76], Lange *et al*^[77] reported that LDLR expression was higher in patients with COVID-19 than in healthy controls. Agirbasli *et al*^[78] reported that LDLR-related protein 1, a member of the LDLR family, is increased in severe COVID-19. According to current findings, angiotensin II inhibits proprotein convertase subtilisin/kexin type 9 (PCSK9)^[36]. PCSK9 disrupts LDLR^[36]. Angiotensin II indirectly increases LDLR levels. In severe cases of COVID-19, increased angiotensin II may lead to NHE overstimulation and lipid accumulation in the liver. Besides, the virus needs cholesterol particles to form new virions after infecting cells^[79]. Since the liver is the production and storage site of cholesterol, the virus can continue its life cycle in the liver for a lengthy time. Some studies have shown that high-density lipoprotein (HDL) binds to SARS-CoV-2^[80]. Low HDL facilitates the virus binding to ACE2^[80]; however, high HDL may play an active role in SARS-CoV-2 transfer from other infected tissues to the liver, as HDL is a cholesterol transporter of the liver.

The virus may settle in the liver and cause hepatocyte damage and multisystem disorder. Therefore, liver involvement may contribute significantly to mortality in COVID-19. There may be a close relationship between liver injury and fibrinogen and coagulation disorders in patients with COVID-19^[3]. As we know, fibrinolysis increases liver necrosis^[81]. Initially, fibrinogen and D-dimer levels are elevated in patients with severe COVID-19, but their levels decrease over the following days^[82]. Fibrinogen is an acute phase reactant primarily released from the liver, and IL-6 increases fibrinogen synthesis^[83]. Since the release of proinflammatory cytokines such as IL-6 increases with NHE activation, the virus may indirectly cause an increase in fibrinogen and D-dimer. In severe cases of COVID-19, fibrinogen and D-dimer levels were found low after ten days. NHE increases the tendency to thrombosis mainly through platelets^[84]; however, it may also indirectly lead to a tendency to thrombosis through the liver. Prothrombin

synthesized in the liver is converted to thrombin when fibrinogen is transformed into fibrin. Thrombin levels are high in patients with COVID-19^[85]. Thrombin activates NHE in vascular smooth muscle cells^[86]. Thus, the virus can cause damage to distant organs with the help of NHE-mediated proinflammatory cytokine release and thrombin.

While the virus causes a Na^+ load in the cell by NHE overstimulation, it overloads H^+ in the extracellular area^[4]. As a result of the subsequent redox reactions, H^+ flows into the cell, and excessive ATP consumption by NKA results in hypoxia and ATP depletion in the cell^[12]. Hypoxia and ATP depletion are prominent stimuli of heme oxidase^[87,88]. Increased heme oxidase activation enhances carbon monoxide (CO) and bilirubin levels in the cell^[89]. CO usually has a hepatoprotective effect, but sometimes it can also have a hepatotoxic effect^[88]. Bilirubin increase in patients with COVID-19 has been identified as a predictive marker for mortality rates^[90]. Bilirubin elevation may indirectly reflect NHE-mediated cell hypoxia in patients with COVID-19.

The elevation of ALP and GGT in COVID-19 patients suggests that SARS-CoV-2 changes the structure of bile acid and may lead to cholestasis^[91]. NHE_1 on the basolateral surface of the bile ducts regulates intracellular pH, while NHE_3 on the apical surface regulates bile acid structure and fluidity^[92]. Increasing NHE_3 activity leads to Na^+ loading, which impairs the containment and fluidity of bile acid^[32]. Bile acids play a primary role in maintaining gut microbiota composition^[93]. We have recently described that disruption of NHE-mediated gut microbiota composition leads to cytokine release syndrome^[12]. The virus disrupts the gut microbiota composition in the intestine and increases the release of proinflammatory cytokines like $\text{TNF-}\alpha$. In addition, SARS-CoV-2 increases local angiotensin II levels by binding to ACE2. Unlike other tissues, $\text{TNF-}\alpha$ and angiotensin II decrease NHE_3 activity in the intestine^[12,94,95]. Thus, increased luminal Na^+ leads to diarrhea and cytokine release^[12,96]. This event may play a role in triggering cytokine storms^[12]. The virus can cause intestinal-associated cytokine release by changing the structure and fluidity of bile acid before it has intestinal involvement. It is not yet known whether the changed content of bile acid will play a role in transferring the virus to the intestine. Detailed studies are needed on this subject.

CONCLUSION

Physiological NHE activation protects the liver against acute injury, whereas NHE overstimulation causes severe liver injury. NHE overstimulation can lead to hepatocyte death. Also, it increases the urea cycle and inhibition of gluconeogenesis and vitamin D synthesis in the liver. NHE overstimulation may cause thrombotic events and systemic damage by increasing fibrinogen levels and cytokine release. It can also change the gut microbiota composition by disrupting the structure and fluidity of bile acid, thus triggering systemic damage. SARS-CoV-2-caused local and systemic damage can be improved by preventing the virus-induced NHE overstimulation in the liver.

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