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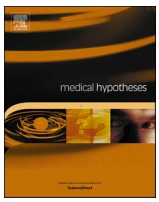

Insulin may promote SARS-CoV-2 cell entry and replication in diabetes patients

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ABSTRACT

Patients with diabetes often have severe hyperglycemia triggered by novel coronavirus disease 2019 (COVID-19). Insulin treatment should be the main approach to the control of acute hyperglycemia in patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. However, clinical investigation found that insulin treatment is associated with a significant increase in mortality risk in patients with diabetes and SARS-CoV-2 infection. The reason for this high mortality rate remains obscure. Previous studies have demonstrated that insulin is an activator of Na^+/H^+ exchanger (NHE) which could decrease extracellular pH and increase intracellular pH and glycolysis. Here, the author emphasizes insulin may contribute to SARS-CoV-2 cell entry and multiplication in host cells through activation of Na^+/H^+ exchange. Additionally, the inhibition of Na^+ /H^+ exchange activity or glycolytic flux can result in reduced mortality in patients with COVID-19 and diabetes mellitus during insulin treatment.

Introduction

The current pandemic of COVID-19, caused by SARS-CoV-2 infection, is a particular challenge for patients with diabetes [1]. COVID-19 predisposes patients to severe hyperglycaemia because of insulin resistance and impairments of both insulin production and secretion from pancreatic β-cells [2]. The results from a multi-centered study showed that hyperglycaemia is associated with worse outcomes in patients with COVID-19, and SARS-CoV-2 infected patients with well controlled blood glucose have better treatment outcomes than those with poorly controlled glycemia [3]. People with diabetes mellitus receive insulin therapy as part of their treatment to achieve blood glucose control. Diabetes patients with COVID-19 need more insulin to maintain blood glucose at a constant level [2]. Furthermore, the clinical investigation showed that insulin treatment is associated with higher mortality compared to other anti-diabetic therapy in patients with COVID-19 and diabetes [4]. Another study also demonstrated that higher inpatient insulin requirements are associated with a higher mortality rate and higher need for intubation therapy [5]. Insulin treatment is associated with several side effects, such as hypoglycemia, inflammatory and vital organs damage, which are suspected to be contributors to the increased mortality in patients with COVID-19 [4,5]. However, the mechanism underlying the association between insulin treatment and poor outcomes for patients with COVID-19 and diabetes is still relatively little known. The possible reasons behind this phenomenon are multifaceted.

It has been postulated that an increase in Na^+/H^+ exchange activity may contribute to SARS-CoV-2 infection and prolonged Na^+/H^+ exchanger (NHE) activation may aggravate COVID-19 severity in patients [6]. NHE exists on the plasma membrane of cells. The main function of NHE is the electroneutral exchange of extracellular Na^+ for intracellular protons, which plays an important role in the precise regulation of cellular pH and volume. The NHE is not only activated by the acidification of the cytosol, but also activated by growth factors, hormones and hyperosmotic stress [7]. Previous studies have demonstrated that insulin affects NHE in a variety of ways, for instance, insulin directly stimulates Na^+/H^+ exchange activity [8], increases the expression of NHE and induces NHE translocation on the cell surface [9,10]. Based on the above viewpoints, it is reasonable to speculate that insulin treatment could enhance SARS-CoV-2 infection in patients. Here, I would like to mention the effects of insulin on the SARS-Cov-2 cell invasion and replication by activating Na^+/H^+ exchange pathway.

Insulin promotes viral entry into host cells

Previous studies showed a low pH environment plays an important role in the infections of coronaviruses including MERS-CoV, SARS-CoV and SARS-CoV-2 [11]. There are two routes of cell entry for viruses, one is direct fusion at the host cell surface, the other is fusion following

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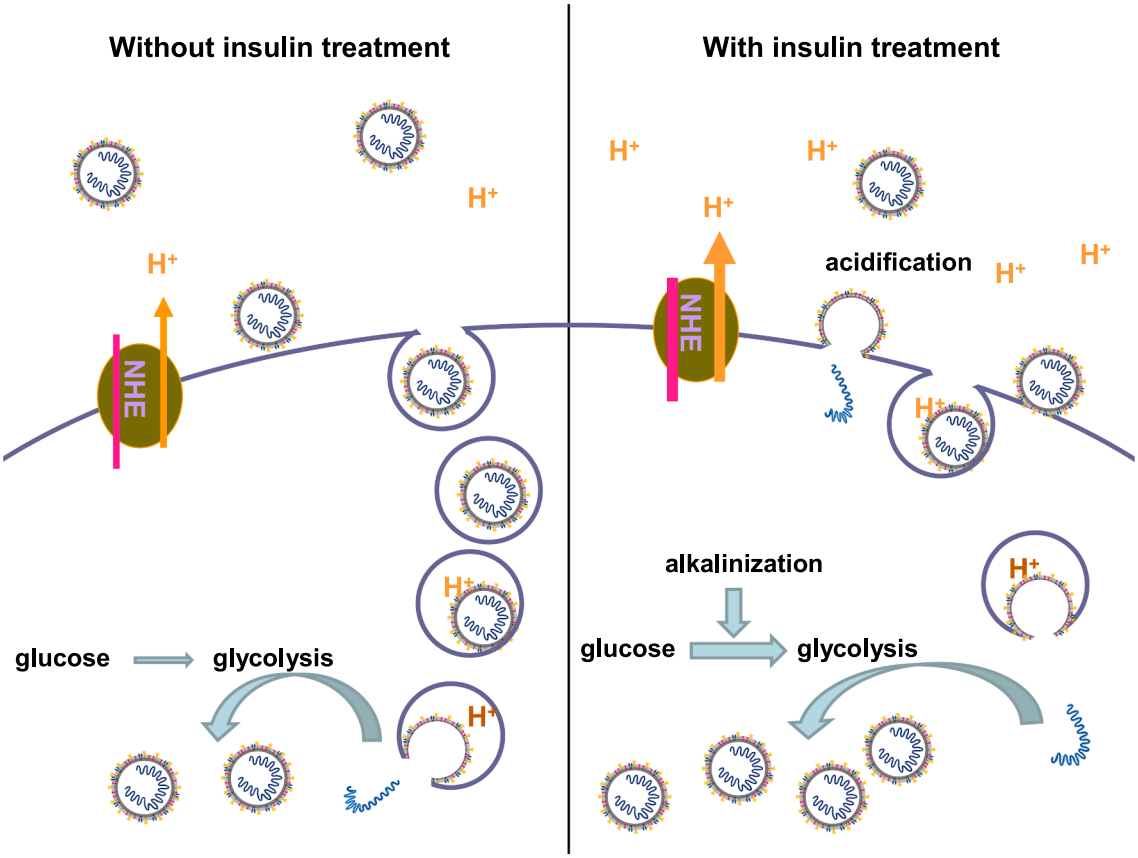

Fig. 1. The difference of SARS-CoV-2 cell entry and replication between with insulin and without insulin treatment.

endosomal uptake. First, the fusion between viral and cellular membrane is a direct route for SARS-CoV-2 cell entry. Recent research confirms an acidic condition is essential for membrane fusion and release of viral genome into the host cell [12]. Next, the SARS-CoV-2 spike binds its mobile receptor-binding domains (RBDs) to the host cell angiotensin-converting enzyme 2 (ACE2) receptor for facilitating virus entry, which is through low-pH-endosomal pathways [13]. Both routes require virion exposure to an acidic environment for successful infection. Insulin, as a hormone stimulator of NHE, can increase intracellular Na^+, which is correlated with efflux of H^+ [8]. Furthermore, NHE actively extrudes proton to extracellular environment which leads to extracellular acidification [14]. It is worth noting that insulin enhances Na^+/H^+ exchange activity in a time- and concentration-dependent manner [15]. At addition, insulin requirements are increased in patients with hyperglycaemia during severe SARS-CoV-2 infection [3]. Therefore, the excessive and continuous activation of NHE induced by insulin can permanently keep relatively low extracellular pH which may facilitate SARS-CoV-2 cell entry and infection. Perhaps this is one reason why insulin treatment associates with higher mortality compared to other anti- hyperglycemic agents in patients with COVID-19 and diabetes.

Insulin promotes viral replication

Many viruses, including human cytomegalovirus, SARS-CoV-2, rhinovirus, Epstein-Barr virus and adenovirus, alter the host cell metabolism to glycolysis for their replicative advantage. The metabolic alterations produce rapid energy and a carbon source for the synthesis of nucleotides, amino acids and lipids to meet the needs of viral replication [16]. Further researches found the SARS-CoV-2 infection evokes mitochondrial ROS production which enhances stabilization of hypoxia-inducible factor-1α (HIF-1α) and consequently promotes glycolysis for SARS-CoV-2 replication [17]. Moreover, 2-deoxy-D-glucose (2-DG), a glycolysis inhibitor, can prevent SARS-CoV-2 replication in caco-2 cells [18]. In contrast, oligomycin, an inhibitor of ATP synthase, promotes viral replication in human monocytes through increase in glycolytic flux and decrease in ATP synthesis. Thus, glycolysis is necessary for sustaining SARS-CoV-2 replication [17]. Accumulating evidence indicates that the rate of glycolysis is augmented by an increase in intracellular pH [19,20]. Studies in frog skeletal muscles and rat adipocytes have shown that insulin stimulates Na^+/H^+ exchange and the resultant intracellular alkalinization is involved in the stimulation of glycolysis [8,21]. Then, insulin may promote SARS-CoV-2 replication through an increase in intracellular alkalinization and glycolysis. The mechanism that insulin increases viral load or persistence maybe is another reason of worse outcomes in patients with diabetes and COVID-19.

Hypothesis and test

The hypothesis is that an increase in Na^+/H^+ exchange activity induced by insulin contributes to SARs-CoV-2 entry and replication in host cells. Insulin-enhanced Na^+ /H^+ exchange activity decreases extracellular pH which facilitates SARS-CoV-2 cell entry, and increases intracellular pH and glycolysis which promotes SARS-CoV-2 replication in cells, respectively (Fig. 1). In order to test the above hypothesis, basic study is needed to make clear that the effect of insulin on the SARS-CoV-2 entry and replication in the host cells. Clinically, the viral load and persistence in patients with COVID-19 should be compared between insulin and non-insulin anti-diabetic treatment. According to the reported method [22], viral load and persistence can be examined in samples including sputum, serum and urine from the patients with COVID-19. The viral load is detected by real-time quantitative reverse transcription-PCR assay and assessed using viral RNA concentration in the sample from patient. Viral persistence is calculated as the number of days from symptom onset or viral positivity to viral disappearance. The

peak value of viral load and viral persistence may be major indicators for therapeutic efficacy study.

The possible explanations for clinical phenomena

Research results confirm that the glycolysis is both necessary and sufficient for SARS-CoV-2 replication. At addition, elevated glucose levels directly increased viral load, ACE2, and proinflammatory cytokines expression in SARS-CoV-2-infected monocytes in a dose-dependent manner, which suggests the more entry of glucose into cells, the more SARS-CoV-2-stimulated glycolytic flux and viral replication [17]. Although insulin can effectively decrease blood glucose levels, it increases the transport of glucose into cells and glycolysis through Na^+/H^+ exchange activity [21]. Therefore, we cannot exclude that insulin provides an extra favor for SARS-CoV-2 replication. Because some of diabetes patients receive insulin therapy to control blood glucoses, the preexistence of insulin therapy maybe already creates an advantage for SARS-CoV-2 infection. Thus, this hypothesis might provide partial explanation for clinical phenomena including susceptibility to COVID-19 infection, adverse outcomes [3], especially the phenomena that blood glucoses is controlled well prior to coming into the hospital without a better prognosis and insulin treatment is associated with increased mortality risk in patients with COVID-19 and diabetes [4,5].

The possible clinical implications of hypothesis

Based on hypothetical mechanism, the possible treatment strategies will be talked about. Firstly, when higher load or longer persistence of virus occurs in patients with insulin therapy alone, non-insulin lowing glucoses drugs or combinations of insulin and other anti-diabetic agents should been taken into account. Secondly, hyperglycaemic state can last for more than two months in patients who recovered from COVID-19 [23]. Accordingly, when SARS-CoV-2 cannot be detected in patients, insulin may be reused for the treatment of hyperglycaemia. At last, insulin plays a key role in glucose uptake and oxidation in cells to meet energy requirement for their multiple functions. In clinical practice, insulin therapy is an indispensable in the treatment of patients including type 1 diabetes patients, some of type 2 diabetes patients and patients with server side effect of non-insulin hypoglycemic drugs. According to above hypothesis, the inhibition of NHE activity could improve pH microenvironments of both sides of cell membrane, and resultantly decrease severity of SARS-CoV-2 infection during insulin treatment. Perhaps, NHE inhibitors, such as amiboride, maybe are beneficial for patients with insulin therapy. A more direct approach is to elevate endosomal pH and concomitantly inhibit SARS-CoV-2 infection because endosomes undergo rapid acidification due to extracellular acidic environment, and low endosomal pH results in release of viral contents into the cell [12]. Further study showed endosomal acidification inhibitors including niclosamide and bafilomycin A1 could block SARS-CoV-2 infection [11,12]. Therefore, neutralization of acidic endosomes maybe is a potential strategy to control SARS-CoV-2 infection in patients with insulin treatment. In addition, glycolysis inhibitors such as 2-DG, could be considered to prevent SARS-CoV-2 replication during insulin therapy. In sum, the hypothesis and methods need to be further tested. If confirmed, these methods could bring better outcomes for the patients with insulin therapy during SARS-CoV-2 infection.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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