





Oral Delivery of ACE2 Bioencapsulated in Plant Cells as Potential Adjuvant Therapy to Reduce the COVID-19 Disease Severity

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Abstract: COVID-19 has become a widespread pandemic and a devastating public health emergency. Numerous trials have been conducted to search beneficial therapeutical interventions. ACE2 plays an essential role in the pathogenesis of SARS-CoV-2 infection as this receptor become the entry point of virus to the cell. The blockage of the ACE2 receptor and the delivery of ACE2 in the soluble form are some mechanisms that have been proposed for potential therapy of COVID-19. Parenteral administration of ACE2 in soluble form has been conducted in trials using the hrACE2 (human recombinant ACE2) and showed a favorable result. However, the possibility of administering ACE2 through an oral route has not been extensively explored. Bioencapsulated plant cell technique has shown resistant to digestive enzymes and gastric acid and able to carry ACE2 to be absorbed safely into the circulation. Previous study showed promising utilization of ACE2/Ang1-7 Bioencapsulated in Plant Cells to treat ocular inflammatory disorders in mice. Although no clinical studies have been done yet, similar concept can be theoretically applied to hinder the development of SARS-CoV-2 severe manifestation. The increasing soluble ACE2 may reduce the circulatory levels of detrimental Angiotensin II effects as well as acting as a decoy to bind free virions from attaching to the target cells.


1 INTRODUCTION


COVID-19 has become a global public health emergency with the increasing emergence of new cases daily in countries worldwide. There is an urgent need for therapeutics now, more than ever, to control SARS-CoV-2 infection (Vellingiri et al., 2020). The biomedical community has made a massive effort to find potential drugs by conducting many trials in search of an effective cure for COVID-19. (The Lancet Infectious Diseases, 2020). ACE2 activator, especially in the soluble form, is deemed one of the plausible therapeutic strategies to control SARS-CoV-2 infection, for this receptor acts as a gateway of SARS-CoV-2 infection and the foundation of the pathogenesis of COVID-19. This soluble form of ACE2 can act as a competitive interceptor of SARS-

CoV-2 by binding the virus particles and limiting the virus's attachment to the host's cell membranes (Battle et al., 2020; Rodríguez-Puertas, 2020). Parenteral administration of human recombinant soluble ACE2 (hrsACE2), has been tested in patients and has passed phase 2 clinical studies with a great safety profile. A recent study by Zoufaly et al. (2020) has shown that the administration of parenteral hrsACE2 to a patient with severe COVID-19 resulted in marked reductions in SARS-CoV-2 viral load, serum levels of inflammatory cytokine, and serum levels of Angiotensin II of the recipient (Abd El-Aziz et al. 2020). The route of administration of soluble ACE2 through oral route has not been explored extensively, despite successful trials of oral ACE2 bioencapsulated plant cell in animal studies with favorable results (Shil et al., 2014). In this study, we explore the possibility and feasibility of oral bioencapsulated ACE2 in plant cells as a potential adjuvant therapy to ameliorate COVID-19 manifestations in humans and how this drug would potentially revolutionize the pharmaceutical industry,

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especially in developing countries all over the globe, through its advantageous cost-efficiency.

2 BACKGROUND

2.1 SARS-CoV-2 Infection and ACE2 Molecular Pathogenesis

SARS-CoV-2 are single-stranded RNA viruses and contain two groups of proteins, namely structural protein such as spike (S) proteins that bind to the receptors on the host cell, nucleocapsid (N) that protects the genetic information of virus, matrix (M) and envelope (E), and non-structural proteins such as proteases (nsp3 and nsp5) and RdRp (nsp12) (Chatterjee et al., 2020). SARS-CoV depends upon ACE2 receptors expressed in human epithelial cells, endothelial cells, and most abundantly in the lung parenchyma. This receptor recognition is one of the significant steps in viral infection of host cells and the prelude of its pathogenesis (Chatterjee et al., 2020).

The S protein in the SARS-CoV-2 membrane promotes virus entry into the host cell through ACE2 receptors widely spread among many cells in the various organ system. Despite ACE2's existence in various cells, 83% of total ACE2 in humans is expressed in alveolar epithelium type 2, making this virus's prominent tropism in the respiratory system. Typically found in membrane-bound form, ACE2 comes in a smaller fraction in the soluble form (Verdecchia et al., 2020). SARS-CoV-2 entry to the cell will cause a depletion of ACE2 expression. ACE2, which functions in cleaving AngII (Angiotensin II) into Ang1-7 (Angiotensin 1-7), helps regulate the balance of the two. An increase of AngII due to deficiency of ACE2 will promote pro-inflammatory, pro-oxidative, pro-fibrotic processes, and vasoconstriction, which cumulatively contribute to the deterioration of COVID-19 manifestations (Zhang et al., 2020).

AngII plays a vital role in signaling cellular and molecular events critical in the pathogenesis of pulmonary fibrosis. The first mechanism is to promote pro-inflammatory cytokines such as IL-6 and IL-8 by macrophages; the second is by producing reactive oxygen species (ROS) among infected epithelial cells and followed by its apoptosis and lastly by proliferation, migration, and differentiation of fibroblast to myofibroblast. Thus, the higher serum AngII among patients with COVID-19 pneumonia, the higher the risk of developing respiratory failure and other adverse events (Delpino & Quarleri, 2020).

2.2 Bioencapsulated Plant Cell to Deliver Protein-based Drug

Incorporating protein-based drugs into plant cells has become a cutting-edge method called bioencapsulated plant cells through complex biotechnological engineering. There are two methods to make the desired protein expressed in the plant cell, through nuclear manipulation or chloroplast manipulation. The chloroplast manipulation is considered more superior to nuclear manipulation to increase the levels of transgene expression. Each plant contains about 10,000 copies of the chloroplast genomes, and collectively they expressed up to 70% of total leaf protein. A plethora selection of proteins, ranging from minuscule antimicrobial peptides or hormones to large-sized proteins encoded by bacterial, viral, fungal, and human genes, have been successfully expressed in chloroplasts (Kwon & Daniell, 2015).

The genes that optimized the desired protein expression are usually fused with CTB (cholera toxin type B) to facilitate transepithelial transport in the gut. After the fusion, the combined material will be cloned into the chloroplast transformation vectors. Shoots emerging with the modified chloroplast are further investigated by using PCR to confirm the site-specific integration of the chloroplast genome. After the confirmation, the plants will be transferred to the greenhouse for propagation and mass production. The leaves of the plant that contain the desired protein will go through a process called lyophilization. Lyophilized plant cells are stable at a certain range of temperature for many years, can further withstand the digestive enzymes and denaturation from gastric acid, maintaining the protein drug's structure and functions (Park et al., 2020).

Plant cells' walls are composed of sturdy lignin and cellulose, which cannot be broken down by digestive enzymes. Combined with the effects of lyophilization, layers of protection screen protect the desired protein-based drug. After the bioencapsulated drug arrives in the intestinal lumen, the intestinal bacteria, especially from the *Bacteroides* spp. and *Firmicutes* species, will break down lignin and cellulose from the plant's cell wall cell, releasing the drug into the intestinal lumen. In this step, the CTB helps in absorption of the drug by translocating the drug through the gut epithelium. After the absorption, the drug will be released into the circulation (Kwon & Daniell, 2016).

The drug administration technique through bioencapsulated drug in plant cells is considered cost-efficient because it does not need complex cold-chain

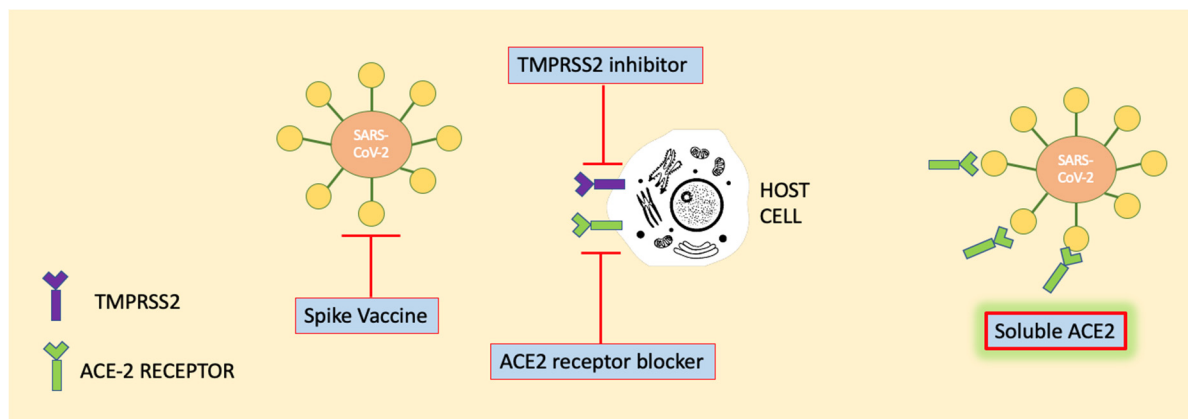


Figure 1: ACE2 as SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic targets.

storage and the drug contained within the plant cells through lyophilization can still be viable for years. Clinical advancement of this concept would revolutionize protein drug production and delivery for many metabolic and genetic disorders (Hu et al., 2020).

2.3 The Potential Capacity of Bioencapsulated ACE-2 As COVID-19 Adjuvant Therapy

This Bioencapsulated technique has been used to treat various diseases from Gaucher's disease, diabetes mellitus, hypertension to Alzheimer's disease. In Gaucher's disease, there is a trial using carrot cells expressing human glucocerebrosidase administered orally to rats, although it was found that the concentration of glucocerebrosidase post-intervention in the serum was 10-fold lower than the control group with the IV formulation. In diabetes mellitus, design for ideal oral insulin through bioencapsulated plant cell is still in the path of development (Kwon & Daniell, 2016). There is also a study by Park et al. (2020) using IGF-1 bioencapsulated in lettuce cells to promote fracture healing that showed promising results. There is still an endless potential of this novel drug delivery system that needs to be explored, and COVID-19 can be one of them.

SARS-CoV-2 enters the cell through the ACE2 receptor facilitated by the S-protein spike of the virus (Li et al. 2020). ACE2 is mostly bound to cell membranes and only scarcely present in the circulation in a soluble form (Verdecchia et al., 2020). The entry of the virus is followed by downregulation of ACE2 (Gheblawi et al., 2020). The downregulation of ACE2 causes the levels of Ang II to rise. The increased levels of AngII create a

detrimental chain of events that support vasoconstriction, pro-inflammatory, pro-oxidative, and pro-fibrotic conditions, leading to acute lung injury in COVID-19 patients. (Lugito et al., 2020; Bourgonje et al., 2019; Kuba et al., 2005). It explains current potential therapeutic strategies to manage SARS-CoV-2 infection, which are portrayed in figure 1, could be achieved by making spike protein-based vaccine, inhibition of transmembrane protease serine 2 (TMPRSS2) activity, blocking ACE2 receptor, and delivering an excessive soluble form of ACE2 (Zhang et al., 2020).

There have been numerous trials concerning the management of COVID-19 through the involvement of the ACE2 receptor. One of them is a trial using rhACE2 (recombinant human ACE2), which was administered parenterally. This drug has completed clinical trials and efficiently lowered plasma angiotensin II and increased angiotensin 1-7 levels, respectively (Gheblawi et al., 2020; Zoufaly et al. 2020). There has not been any study or trials mentioning a possible ACE2 administration through the oral route, but a proposed model uses bioengineered probiotic, *Lactobacillus paracasei*, that secretes soluble ACE2 to help ameliorate COVID-19 manifestations (Senapati et al., 2020). The administration through oral route with bioencapsulated plant cells has never been highlighted as a proposed COVID-19 therapy model.

A study conducted by Shil et al. (2014) used ACE2/Ang-(1-7) Bioencapsulated in Plant Cells, administered orally, as a cost-effective therapeutic strategy for ocular inflammatory diseases in mice. They succeeded in creating ACE-2 fused with CTB in lyophilized bioencapsulated plant cell. The ACE2 activity assay through ELISA (enzyme-linked immunosorbent assay), using protein extracts isolated from plant leaves showed that the plant cells successfully expressed human ACE2 which is

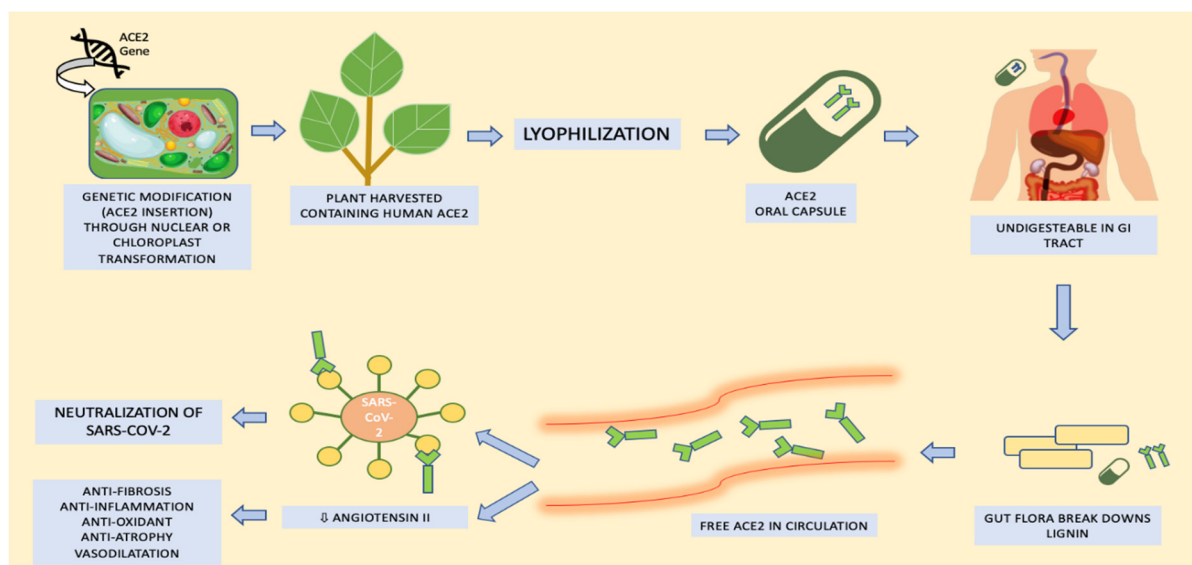


Figure 2: The process of making bioencapsulated ACE2 and its desired effects on SARS-CoV2.

enzymatically active, and they found that the ACE2 protein can be detected in both serum and retina of the mice subjects 5 hours after oral gavage (Shil et al., 2014). There was also a similar study conducted by Shenoy et al. (2014) using oral ACE2/Ang-(1-7) Bioencapsulated in Plant Cells that successfully prevented the progression of monocrotaline-induced pulmonary hypertension in rats.

Despite the successful creation of ACE2 drug bioencapsulated in plant cell for animal studies and how the bioencapsulated protein drug technique has been successfully developed to treat some human diseases, ACE2 drug bioencapsulated in plant cell has never been tested to human subjects. This new drug administration, theoretically speaking, could be one of the therapeutical strategies to deliver a soluble form of ACE2. This ACE2 bioencapsulated plant cell will be released into the circulation right after the cellulose on the plant carrier's outer cell wall is digested by intestinal bacteria. The fusion of ACE-2 with CTB helps to translocate ACE2 into the gut epithelium cells. The ACE2 drug that circulates in plasma can act as a decoy for SARS-CoV-2 binding so that some active viruses might not attach to the ACE2 receptor in various cells in the body. If SARS-CoV-2 already infects the patient, this drug's administration can ameliorate the severity of the infection. Soluble ACE2 has protective effects and can cleave AngII (which brings pro-inflammatory, pro-oxidant, and pro-fibrosis) into Ang1-7 (which has beneficial effects). Thus, through these chains of events, ACE2 that is administered bioencapsulated in plant cells would be able to decrease the severity of COVID-19 infection and lower the incidence of

cytokine storm by regulating the RAS system and keeping the AngII and Ang1-7 in the right balance.

3 CONCLUSIONS

Bioencapsulated ACE2 in plant cells could be a therapeutical strategy to deliver the soluble form of ACE2 in COVID-19 patients. By acting as a competitive interceptor that limits the attachment of SARS-CoV-2 to membrane cells, this soluble ACE2 could prevent SARS-CoV-2 entry and replication in the target cells. Aside from this effect, ACE2 also acts by cleaving AngII, which exerts detrimental properties that aggravate the severity of COVID-19 manifestations. In addition to those benefits, bioencapsulated drug in plant cells is considered cost-efficient because it does not need complicated cold-chain storage, and the ACE2 contained within the plant cells through lyophilization could still be viable for years. Advancement of this bioencapsulated protein drugs in plant cell technique could be just what Indonesia, or other developing countries, need as a potential cost-efficient strategy to ameliorate SARS-CoV-2 infection.

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