Promising renin-angiotensin system treatments in lung diseases

Modulating the renin-angiotensin system in a particular way to cure/treat/prevent a lung disease

Name: Jorn Kreeft
Studentnumber: S4511743
Department: bachelor LST
Date: 23-5-2023
Supervisor: Dr. G.N. Moll
Second supervisor: Dr. J. Broos

Summary

The renin-angiotensin system (RAS) is known for maintaining homeostasis of blood levels and body fluids in humans; recent studies extended that view on the RAS. Further research shows that the RAS is also related to multiple other occurrences in the human body, including organ injury and pathologies of several diseases (Vargas et al., 2022). Thus, the classical RAS ACE/Ang II/AT1R regulatory axis and both the ACE2/Ang-(1-7)/MasR and ACE/Ang II/AT2R counter-regulatory axes are very important to the human body, in general. RAS treatment can prevent and/or treat lung diseases and lung injuries (Tseng et al., 2020). In this thesis, promising RAS treatment for pulmonary fibrosis and COVID-19, two lung diseases, are reviewed.

In the introduction, the key hormone regulation system, RAS, is explained in detail and especially the components of the RAS which are interesting for treatment. Furthermore, two lung diseases/injuries of interest are introduced which are pulmonary fibrosis and COVID-19. Treatment for pulmonary fibrosis is reviewed in more detail whereas treatment for COVID-19 is shortly described. The relation between the RAS in lung tissue and the particular disease is explained. Thus, the role of the RAS in the pathogenesis process of lung fibrosis/COVID-19 is explained and how the RAS is modified after lung fibrosis/COVID-19 pathogenesis. By 'modified' is meant: the different levels of the components of the RAS between healthy circumstances and when infected with the disease. The pros and cons of potential distinct RAS treatments are considered for each particular lung disease.

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Introduction

The renin-angiotensin system (RAS) is a very complex hormonal regulatory system and is a key regulator in the interaction of several organs to regulate multiple body functions. The RAS plays a role in controlling the homeostasis of water, blood, plasma, lymph and interstitial fluid for example (Cheng et al., 2020) (Patel et al., 2017). Initially, research of the RAS was focused on its role in regulating cardiovascular function and related pathologies. New findings in recent studies showed that the RAS is much more complex than researchers thought (Vargas et al., 2022). An imbalance in the RAS axes can result in acute and chronic diseases, including lung diseases. Restoring the balance of the RAS between the detrimental and protective axes can prevent/reduce/partly cure lung injury (Tseng et al., 2020). This is possible by stimulating AT2R or MasR. It is also possible by inhibiting the ACE/Ang II/AT1R regulatory axis, by AT1R inhibitors for example (Tseng et al., 2020). In this thesis I will focus on a few components of the RAS system to target or upregulate for possible treatment against pulmonary fibrosis and COVID-19. The RAS components of interest are: angiotensin converting enzyme 2 (ACE2), Angiotensin 1-7 (Ang-(1-7)), MAS receptor (MasR) and angiotensin II receptor (AT2R) which are the protective components of RAS. RAS inhibitors, which inhibit the ACE/Ang II/AT1R pathway, like AT1R inhibitors and ACE inhibitors, are also considered as possible treatments. Considerations on the pros and cons of existing and upcoming therapeutic approaches for the diseases of interest are being discussed separately.

The renin-angiotensin system

The RAS plays an important role in regulating the blood pressure and body fluid homeostasis but recently the involvement of RAS in the inflammatory responses in kidney, liver cardiovascular system and lungs was unraveled (Tan et al., 2017). Renin, a key component of the RAS, is secreted by the juxtaglomerular epithelioid cells located in the medial layer of renal

afferent arterioles in the kidney, this is where the RAS starts (Tan et al., 2017). The angiotensinogen protein is cleaved by renin and angiotensin I (Ang I) is formed. Ang I is cleaved by angiotensin converting enzyme (ACE) and thereby converted into angiotensin II (Ang II). Ang II exerts its biological effects by activating AT1R and AT2R. AT1R is coupled to a G protein which is able to stimulate multiple signaling pathways. Activation of the AT1R mediates vasoconstriction, inflammation, weak bronchoconstriction and catecholamine release from peripheral sympathetic neurons via NF-κB activation. Reactive oxygen species (ROS) production, apoptosis and lung fibroblast proliferation is also mediated by activation of AT1R (Tan et al., 2017). Binding and activating the AT2R results in vasodilation and growth inhibition, facilitated by the activation of which phosphatases. ACE is responsible for degradation of two local pro-inflammatory and protussive (cough enhancing) peptides, bradykinin and substance P (Tan et al., 2017). These peptides can trigger the release of nitric oxide and prostanoids and the cough reflex is then induced. ACE2 is responsible for the cleavage of Ang II resulting in the formation of Ang-(1-7). ACE2 is just like ACE a zinc metallopeptidase and also a homologue of ACE. Ang-(1-7) is a heptapeptide and targets the G protein-coupled Mas receptor and exerts anti-inflammatory and anti-remodeling effects by stimulating the Mas receptor. The ACE2/Ang-(1-7)/MasR pathway counter-regulates the pro-inflammatory, pro-fibrotic and pro-proliferative effects of the ACE/Ang II/AT1R pathway (Tan et al., 2017).

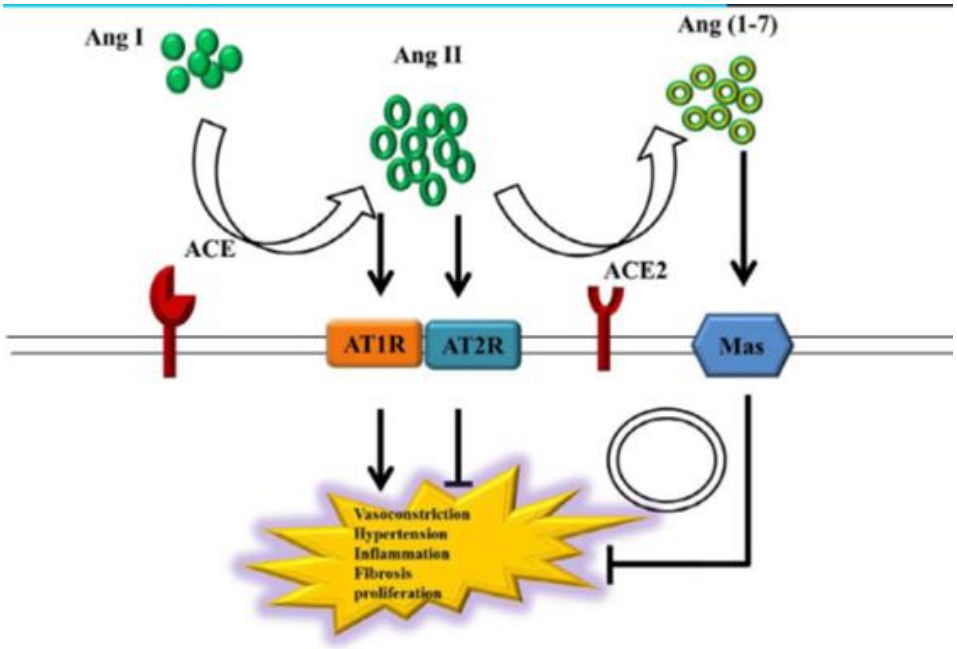

Figure 1: The cleavage pathway in the RAS, starting with the Ang I peptide to get eventually to the Ang-(1-7) peptides. The target receptors of the different angiotensin proteins are identified and also the mediated physiological effects (Tseng et al., 2020). Figure is from (Tseng et al., 2020).

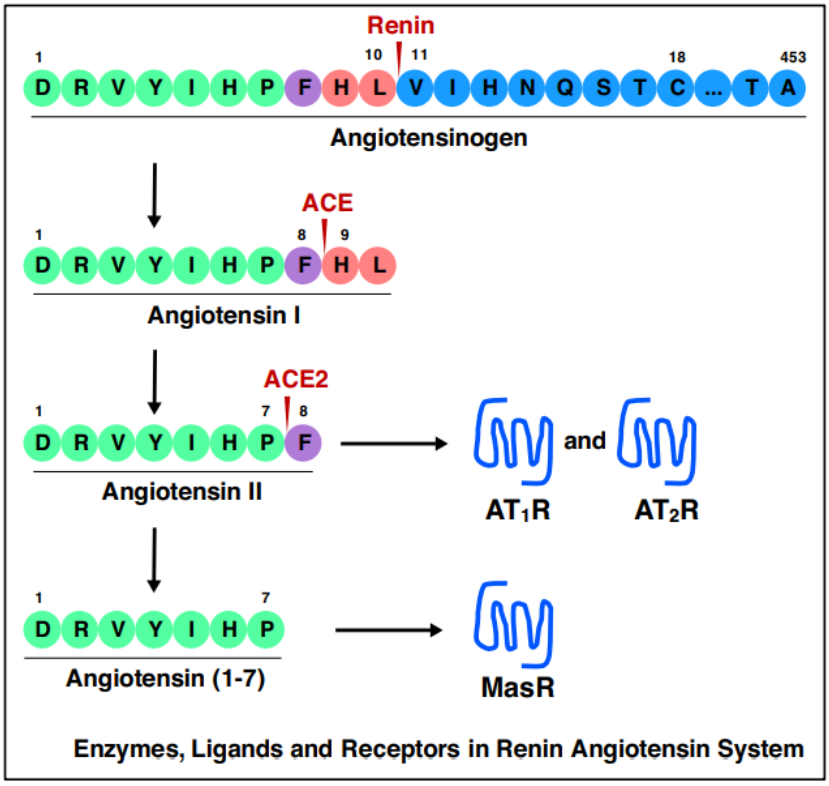

Figure 2: The primary structure of the different angiotensin proteins and which enzyme converts which angiotensin peptide/protein at which location is drawn. ACE also converts angiotensin 1-7 to angiotensin 1-5. ACE2 also converts Ang-(1-10) into Ang-(1-9) which can then be converted by ACE into Ang-(1-7) (Tan et al., 2017). Figure is from (Tan et al., 2017).

The ACE/Ang II/AT1R pathway plays a decisive role in the regulation of vasoconstriction, inflammation, cell proliferation and fibrosis. This pathway is called the classical RAS axis and has a positive role in the regulation of increasing sympathetic nervous system tension, increasing blood pressure, causing vasoconstriction, promotion of inflammation, myocardial hypertrophy and fibrosis (Cheng et al., 2020). The other pathway is the counter-regulatory RAS axis, also called the ACE2/Ang II/AT2R-based pathway. This pathway has a negative role in the regulation so counteracts the classical RAS axis, resulting in an opposite physiological effect (Cheng et al., 2020). Binding of an agonist to the Mas receptor induces comparable physiological effects as the Ang II mediated effect when bound to the AT2R (Cheng et al., 2020). Different components of the RAS system can promote the same physiological effect and the same component of the RAS system can also promote a different physiological effect; this is dependent on to which pathway the specific compound is linked. Ang II can stimulate both the AT1R and the AT2R, depending on which of the two receptors is stimulated, different physiological effects are exerted. Whether AT1R or AT2R is stimulated, depends on the presence of receptor levels and presence of other agonist levels (Cheng et al., 2020). Because of this phenomenon, the body is able to respond coordinately and quickly to stimuli for vasoconstriction and vasodilation (two opposing effects), for example, and is therefore essential in the role of maintaining homeostasis. Stimuli for vasoconstriction and vasodilation can be

picked up from anywhere in the body and is even processed to a small specific local area, together with other RAS related stimuli (Cheng et al., 2020).

Renin

Renin is an acid protease stored and synthesized by secretory vesicles in the juxtaglomerular cells in the kidney. Initially, it is synthesized as prorenin, a proenzyme which has 406 amino acids (Vargas et al., 2022). Prorenin is cleaved into an active renin protein of 340 amino acids and released into circulation. A small proportion of prorenin is also released into circulation to get a renin/prorenin ratio of around 10 in healthy people (Vargas et al., 2022). Plasma renin activity is measured through the concentration of angiotensin I. Renin secretion is induced by stimuli like decreased blood pressure and humoral factors like angiotensin I (Vargas et al., 2022).

Angiotensin I/II

The octapeptide Angiotensin II (Ang II) originates from pre-angiotensinogen, a liver-synthesized precursor 485 amino acid protein (Vargas et al., 2022). Angiotensinogen, a 452 amino acid protein, is formed by enzymatic action in which pre-angiotensinogen is converted into angiotensinogen (Vargas et al., 2022). Ang II also stimulates secretion of angiotensinogen, making it a positive feedback loop. In the circulation, renin and angiotensinogen interact to generate the 10 amino acid peptide angiotensin I (Ang I). Ang I interacts with an angiotensin-converting enzyme (ACE) and angiotensin II is formed. This active 8 amino acid peptide stimulates angiotensin receptors 1 and 2 (AT1R and AT2R) (Vargas et al., 2022). Ang II is degraded by peptidases (amongst others ACE2) and has a half-life of one to two minutes. If Ang II stimulates the AT1 receptors, vasoconstriction, inflammation, fibrosis and cell proliferation can be induced. Except vasoconstriction which is not always organ damaging, these are the organ damaging mechanisms (Tan et al., 2017). If Ang II and/or other AT2R agonists stimulate(s) the AT2R, the damaging physiological effects of AT1R mediated by Ang II are counteracted. In most of the tissues more AT1R is present in comparison to AT2R (Tan et al., 2017). This would result in damaging pathological effects on tissue and not enough of the protecting effect via the AT2R pathway if other protective routes would not work such as those resulting from MasR stimulation (Tan et al., 2017).

Angiotensin-converting enzymes

Angiotensin-converting enzymes have two subtypes, ACE and ACE2. ACE is a key enzyme in RAS, it is a peptidase that converts Ang I into Ang II (Tan et al., 2017). ACE2 is also a key enzyme in RAS and converts Ang II into Ang-(1-7). ACE is a transmembrane protein which is expressed in many epithelial cells, especially in lung tissue (Tan et al., 2017). ACE2 has a single transmembrane segment, N and C terminals which are the catalytically active extracellular domains and an intracellular segment and is therefore a membrane protein (Tan et al., 2017). ACE2 has a single enzymatic active site and ACE has more active sites, this is the characteristic difference between the two (Vargas et al., 2022). ACE2 promotes the conversion

of Ang II to angiotensin 1-7 (Ang-(1-7)), Ang-(1-7) stimulates MasR which mediates an organ protecting effect and furthermore promotes the conversion of Ang I to angiotensin 1-9 (Tan et al., 2017). ACE similarly converts Ang-(1-7) to Ang-(1-5), the ACE subtypes basically convert angiotensin types to other angiotensin types (Tan et al., 2017).

Renin-angiotensin system receptors

There are specific receptors for the RAS components: renin and angiotensin. Renin, extrarenal and renal prorenin activate the renin/prorenin receptor at tissue level. Active renin is generated by the cleavage of prorenin and the active renin can then generate Ang I (Vargas et al., 2022). Binding of renin and prorenin to the renin receptor results in the production of pro-inflammatory cytokines and cell differentiation processes via the intracellular MAPK signaling pathway (Vargas et al., 2022). Angiotensin receptors 1 and 2 (AT1R and AT2R) are the two subtypes that have been described. The receptors are proteins with seven transmembrane domains. The intracellular calcium concentration is increased by AT1R which is a G protein-coupled receptor. AT1R has subtype A and subtype B which are present in different tissues. The genes coding for AT1R are located on chromosome 3 (Vargas et al., 2022). Ang II stimulates the AT1 receptor. AT2 receptors activate membrane potassium channels indirectly via various phosphatases which are activated by the Ang II mediated effect of binding AT2R (Vargas et al., 2022). The regulation of these ion channels is involved in maintaining the vascular tone (Wikipedia 2022). Ang-(1-7), Ang-(1-9), Ang II, Ang A and Ang III target the AT2 receptor (Vargas et al., 2022).

MAS receptors

The proto-oncogene MAS1 encodes for Mas receptors (MasR) which are transmembrane proteins coupled to G proteins. The proto-oncogene has a role in the growth and proliferation of cells (Vargas et al., 2022). A mutation in the proto-oncogene can result in the upregulation of cell growth which induces tumors in animal bodies (Oncogene 2021). Mas receptor activation decreases blood pressure, fibrosis, chronic hypertension and sympathetic tone. Activation of the Mas receptor also increases parasympathetic tone, vasodilation, baroreflex, nitric oxide production and natriuresis (Vargas et al., 2022). The natural ligand for Mas receptors is Ang-(1-7). The MrgD receptor is a Mas related receptor, which shows affinity for alamandine (Vargas et al., 2022). MasR inhibits the Ang II bound to the AT1R mediated effect (Vargas et al., 2022).

Idiopathic pulmonary fibrosis

Pulmonary fibrosis is characterized by fibroblast proliferation, alveolar injury and excessive deposition of extracellular matrix proteins, this lung disease results progressively in respiratory failure and death (Gupta et al., 2021). Angiotensin II, a key vasoactive peptide of the RAS, plays a central role in the pathogenesis and progression of idiopathic pulmonary fibrosis (Gupta et al., 2021). Pro-inflammatory and pro-fibrotic effects on the lungs are induced by stimulation of AT1R (by Ang II). Identification of newly discovered protective counter-regulatory axis and its peptides,

enzymes and receptors of this RAS pathway led to novel concepts and possible treatments (Gupta et al., 2021).

The lungs are the gas-exchange organ and therefore the oxygenator and ventilator of the human body, a part of the lungs is called the alveolar epithelium and plays a key role in pathogenesis of this disease (Gupta et al., 2021). The alveolar epithelium, the primary site of gaseous exchange, is a part of the lung parenchyma. Injury of lung parenchymal evokes a reparative response to restore normal lung function (Gupta et al., 2021). When the injury to the alveolar epithelium is repetitive or protracted, dysregulation of the healing process occurs resulting in irreversible scarring of the lungs (Gupta et al., 2021). Normal breathing is affected when sufficient proportion of the lung becomes damaged, this will lead eventually to respiratory failure and death (Gupta et al., 2021).

The relation between RAS and the disease

The RAS plays an active role in the pathogenesis of fibrotic lung diseases, this is supported by a few findings and I will mention a few important ones of them: progression of idiopathic pulmonary fibrosis (IPF) is associated with polymorphism in the angiotensinogen gene, a direct pathological role for renin has been implicated in IPF development, Ang II binding to AT1R induces oxidative stress and regulates inflammatory signaling pathways in the lungs which contributes to fibrogenesis and last, angiotensin converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB) offer protection against experimental models of lung fibrosis (Gupta et al., 2021). Thus, an altered RAS is associated with lung fibrogenesis. The ACE2/Ang-(1-7)/MasR axis and the ACE2/Ang II/AT2R axis are believed to oppose the actions of Ang II via AT1R and protects against organ damage (Gupta et al., 2021). Renin, ACE, angiotensinogen and Ang II have been identified within the lung tissue and also lung parenchymal cells express AT1 and AT2 receptors (Gupta et al., 2021). Furthermore, ACE2 and the Mas receptors have also been detected in the lungs so a local intra-pulmonary RAS is present and this system plays a vital role in maintaining lung homeostasis. Imbalance in the local RAS contributes to cardiopulmonary disorders like pulmonary fibrosis (Gupta et al., 2021).

Alveolar epithelial cells (AEC) serve as a barrier between the environment and the lung interstitium. these cells come in two types: the alveolar type I and the alveolar type II cells (Gupta et al., 2021). Type I makes up for more than 90% of the alveolar surface and are thin squamous cells, whereas cuboidal type II alveolar cells cover the rest of the alveolar surface (Gupta et al., 2021). Damage to either type I or II AECs is a key event in initiation and progression of lung fibrosis (Gupta et al., 2021). Normal healing is ensured by re-epithelialization of the alveolar wall when lung injury occurs. Re-epithelialization can be impaired by loss of proliferative capacity, increased apoptosis of the type II AECs and ineffectual migration/differentiation lowers the proliferative capacity; lung fibrogenesis ensues in these cases (Gupta et al., 2021). Apoptotic cell death in AECs is likely induced by Ang II. This is identified by cell culture studies: type II AECs which are exposed to excess of Ang II may die, the same happens to primary rat lung AECs. Agents like bleomycin, amiodarone and TNF-α

stimulate AECs to produce Ang II de novo, resulting in apoptosis (Gupta et al., 2021). Furthermore, the molecular mechanism of oxidative stress and inflammatory cytokines have an important role in lung fibrosis. The imbalance between the formation of reactive oxygen species (ROS) and anti-oxidative defense mechanisms contributes to the disease pathogenesis in IPF (Gupta et al., 2021). Nicotinamide adenine dinucleotide phosphate oxidase (NOX) is a key producer of ROS in the lungs and Ang II is a potent activator of NOX (Gupta et al., 2021). ROS induced by Ang II activates kinases and transcription factors to induce inflammatory cytokine gene expression such as interleukin-1 and IL-6 (Gupta et al., 2021). These cytokines regulate the fibrotic process in IPF via a few mechanisms: fibroblast proliferation, chemoattraction, accumulation of ECM proteins and parenchymal damage (Gupta et al., 2021). Furthermore, Ang II stimulates the AT1R and transcription of transforming growth factor-β (TGF-β) is induced, TGF-β is a cytokine of which sustained production is linked to development of lung fibrosis (Gupta et al., 2021).

Myofibroblast is another key cell type that is actively involved in lung fibrogenesis. Fibroblasts and myofibroblasts have different functions and Ang II can change the fibroblast phenotype to myofibroblast by itself or by secretion of TGF-β and connective tissue growth factor (CTGF) (Gupta et al., 2021). TGF-β and CTGF induce epithelial-mesenchymal transition, which is a process very important to pulmonary fibrosis (Gupta et al., 2021). Ang II signaling is augmented by TGF-β and AT1R expression is increased in human lung fibroblasts. The above-mentioned processes mediate lung fibrosis (Gupta et al., 2021).

Possible RAS treatment against pulmonary fibrosis

A three-drug combination of azathioprine, N-acetyl-cysteine and prednisone was the mainstay of IPF treatment until this triple-therapy approach was shunned after the PANTHER-IPF trial, increased risk of patients' death and serious adverse events were reported (Gupta et al., 2021). Two new drugs were approved for IPF therapy, pirfenidone and nintedanib, but these drugs fail to increase the life expectancy and are associated with severe adverse effects (Gupta et al., 2021). New treatment is needed and since RAS is known to play an active role in pathogenesis of fibrotic lung diseases, RAS therapy is promising. ACE2, Ang-(1-7), MasR and AT2R are the protective components of RAS to enhance and/or stimulate to get an effective treatment for IPF (Gupta et al., 2021). Possible therapeutic alternatives related to the RAS have been proposed and one of the first targets to inhibit was ACE, in the form of ACE inhibitors (ACEis). Captopril, lisinopril, enalapril and fosinopril are ACEis and are already incorporated into clinical practice; these ACE inhibitors are considered as the first line of treatment. These drugs selectively inhibit the RAS, however these ACEis have side effects (Gupta et al., 2021). The therapeutic options have been expanded by AT receptor blockers (ARBs) which were incorporated into clinical practice a few decades later. Irbesartan, valsartan and losartan are ARBs (Gupta et al., 2021). Direct renin inhibitors, DRIs (enalkiren, remikiren and aliskiren), seem to be possible treatments to pathologies that involve elevated renin levels (Gupta et al., 2021). The above-mentioned possible treatments are focused on the blocking of the actions of the classical RAS, now the alternative RAS is discovered, scientists also started working on drugs which can stimulate this

system. Alternative RAS agonists have potential anti-proliferative, anti-fibrotic and anti-inflammatory actions (Gupta et al., 2021). One of the alternative RAS agonists is recombinant human soluble ACE2 which has multiple positive and protective effects (Gupta et al., 2021).

ACE2

ACE2 exerts protective actions against pulmonary fibrosis. People with PF have lower mRNA levels, enzymatic activity and proteins of ACE2 in experimental models (Gupta et al., 2021). Also shown is that gene knockdown or competitive inhibition of ACE2 results in increased levels of Ang II and decreased Ang-(1-7) levels (Gupta et al., 2021). Animals treated with recombinant ACE2 proteins exhibit decreased fibrosis and therefore improved survival (Gupta et al., 2021). ACE2 also protects pulmonary endothelial cells against cell death, pro-apoptotic proteins are decreased and expression of anti-apoptotic proteins is increased (Gupta et al., 2021). Furthermore, ACE2 inhibits EMT in AECs. PF is also attenuated in experimental models of lung injury by synthetic ACE2 activators, genetically modified stem cells which overproduce ACE2 and pulmonary overexpression of ACE2 (Gupta et al., 2021). Soluble ACE2 (sACE2) is formed by proteolytic cleavage by endopeptidase and remains catalytically active and is therefore also protective against lung fibrosis (Gupta et al., 2021).

Soluble recombinant human ACE2 (APN01):

Pros:	No dose-limiting toxicity	Well-tolerated	Positive effect on RAS components	Aerosol intake/oral intake
Cons:	Repetitive injections	Bad stability	Cost of manufacturing	

Table 1: pros and cons of soluble recombinant human ACE2 treatment APN01. Information from (Gupta et al., 2021).

A con was infusion of the treatment in the bloodstream by a needle was the only way of drug administration but aerosol intake of the treatment is also a possibility now (Shoemaker et al., 2022). This is way more comfortable and doable for the patients. APN01 gets directly to the target organ and eventually is taken up by the lung tissue and executes its protecting roles. As already explained in section sACE2 in plant cells, oral intake is also possible (Gupta et al., 2021).

ACE2 activators

Since ACE2 proteins are difficult to store, have a bad stability, need to be repeatedly injected and are expensive to manufacture, there is a barrier to use the protein as medical therapy (Gupta et al., 2021). Development of synthetic molecules that can activate endogenous ACE2 proteins by upregulation of ACE2 RNA like ACE2 activator compounds are an alternative treatment (Gupta et al., 2021). Two potential ACE2 activators are identified by structure-based drug design: XNT and diminazene aceturate (DIZE). In vitro and in vivo studies showed that the

ACE2 activity is enhanced by these compounds and protective actions are rendered against experimental models of human diseases (Gupta et al., 2021).

XNT:	Pros:	Enhances pulmonary vasorelaxation	Prevents maladaptive right ventricular remodeling	
	Cons:	Unfavorable pharmacokinetic properties	Poor water solubility	Acidic pH for solubilization
DIZE:	Pros:	Inhibiting lung proinflammatory cytokines	Decreasing myocardial collagen accumulation	
	Cons:	Mutagenicity	Organ toxicity	

Table 2: The pros and cons of ACE2 enhancers XNT and DIZE. Information from (Gupta et al., 2021).

XNT and DIZE need to be structurally modified to create drug candidates that are less toxic, safe and that have a good pharmacokinetic profile (Gupta et al., 2021).

Chemically modified ACE2 RNA can be used to express therapeutic proteins; prominent ACE2 protein translation and enzymatic activity is achieved (Gupta et al., 2021). Lipid-based formulation of chemically modified ACE2 RNA for pulmonary delivery resulted in strong protein expression selectively in the lungs (Gupta et al., 2021). Advantages of this technique are: There is no risk of insertional mutagenesis and protein therapy can be fine-tuned as per individual patient's requirements due to the self-limited translation of the RNA (Gupta et al., 2021).

Ang-(1-7)

Ang-(1-7) is one of the enzymatic products of ACE2, next to e.g. Ang-(1-9) and also shows protective effects against lung fibrosis by stimulating the MasR (Gupta et al., 2021). The angiotensin heptapeptide counterbalances the organ-damaging effects of Ang II-mediated stimulation of AT1R (Gupta et al., 2021). Overexpression of Ang-(1-7) attenuates pulmonary fibrosis in the bleomycin-model of lung injury by lentiviral-mediated gene transfer (Gupta et al., 2021). Stable long-term transgene expression in tissue is allowed by lentiviral vectors. Lentiviral vectors are ideal gene delivery vehicles for therapeutic applications and research (Funke et al., 2008). Ang II-induced apoptotic resistance of lung fibroblasts via AT1R is inhibited (Gupta et al., 2021). In vitro experiments showed that treatment with Ang-(1-7) prevented death of AECs and Ang-(1-7) inhibits EMT in AECs induced by TGF-β (Gupta et al., 2021). Ang-(1-7) plays a critical role in prevention of lung fibrosis (Gupta et al., 2021).

Ang-(1-7) peptides do have the therapeutic potential to combat lung diseases but the heptapeptide has a short plasma half-life and limited oral bioavailability so an unfavorable pharmacokinetic profile (Gupta et al., 2021). A modified or formulated Ang-(1-7) peptide must be developed to enhance drug stability, oral absorption and half-life duration. Complexation of angiotensin-(1-7) with hydroxypropyl beta-cyclodextrin leads to protection of the peptide against intestinal degradation following oral delivery; the compound also produced beneficial effects against experimental models of cardiovascular disease (Gupta et al., 2021). Furthermore, an active and stable cyclic analog of Ang-(1-7) with resistance to proteolytic cleavage by peptidases has been generated, whose stability and activity are also increased (Gupta et al., 2021).

MasR

Clinical and experimental research highlight that the Mas receptor (MasR) is significant in several physiological processes and disease conditions (Gupta et al., 2021). When Ang-(1-7) binds this receptor, tissue remodeling and lung endothelial barrier function are stimulated and lung inflammation is inhibited. Knockdown of MasR results in the death of AECs (Gupta et al., 2021). Increased expression of AT1R and reduced expression of MasR are characteristics for lungs of patients with IPF (Gupta et al., 2021).

20-Hydroxyecdysone, a steroid hormone, activates the MasR (Gupta et al., 2021). A pharmaceutical grade oral formulation of 20-hydroxyecdysone, sarconeos (BIO101), is being developed. The MasR agonist, BIO101, has a high specificity towards the MasR and does neither affect the AT1R nor the AT2R (Gupta et al., 2021). Furthermore, this MasR agonist exerts antifibrotic, antihypertensive, anti-arrhythmogenic and vasorelaxant properties (Gupta et al., 2021).

COVID-19

A highly pathogenic and transmittable viral infection which emerged in Wuhan (China) spread around the world and caused a global problem in no time. The coronavirus disease (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and is phylogenetically related to other acute respiratory syndrome-like bat viruses (Shereen et al., 2020). First it was thought that these viruses only infected animals until the world witnessed a SARS outbreak which led to the epidemic.

The coronavirus gets its name from the crown-like spikes on the outer surface of the virus. Coronaviruses are 65 to 125 nanometer in diameter and contain single-stranded RNA as nucleic material, ranging in size from 26 to 32kbs (Shereen et al., 2020). SARS-CoV-2 has a high transmission rate, this could be because of a genetic recombinant event at the S-protein in the receptor-binding domain (RBD) region on the viral lipid layer of the virus (Shereen et al., 2020). This genetic recombinant event at the S-protein in the RBD region resulted in a high

binding affinity to ACE2 which the virus uses as a receptor (Shereen et al., 2020). ACE2 is an important component of RAS.

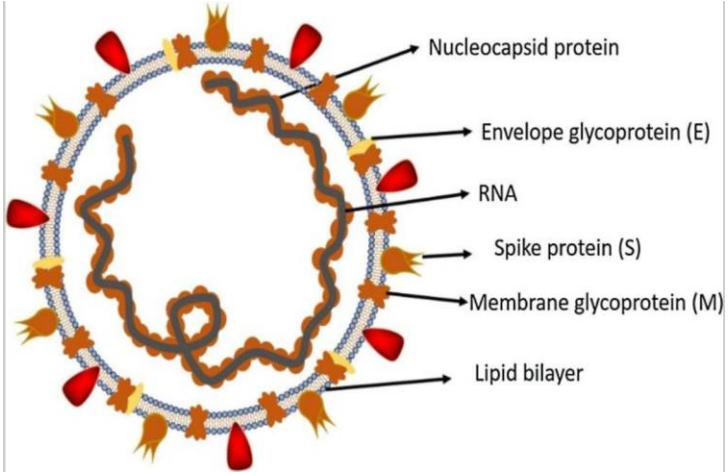

Figure 3: SARS-CoV-2 particles which leads to the COVID-19 disease. All the components of the particle are named even the spike protein (S), which plays an immense role in SARS-CoV-2 infection. Information and figure from (Shereen et al., 2020).

RAS and COVID-19

Summarizing, what happens is that the S-protein of the coronavirus particle targets a specific receptor, ACE2, on target host cells (Tseng et al., 2020). Via this process the virus particles enter the host cells, replicate and cause infection (Tseng et al., 2020). S1 and S2 are the functional units of the S-protein: S2 is responsible for the fusion with host cell membranes and S1 binds directly to the ACE2 receptor since S1 contains the receptor-binding domain (RBD) (Tseng et al., 2020) (Shereen et al., 2020). ACE2 is the host receptor of SARS-CoV-2. S1 binds the ACE2 receptor on the target cell which exposes the cleavage site of S2 and a host protease cleaves of the cleavage site of S2 (Tseng et al., 2020) (Shereen et al., 2020). This process is key for virus infection. ACE2 includes a N-terminal peptidase domain and C-terminal collectrin-like domain, the N-terminal peptidase domain of ACE2 provides the direct binding site for the S-protein of the coronavirus (Tseng et al., 2020). Individuals infected with SARS-CoV-2 have higher circulating Ang II levels compared to healthy individuals, SARS-CoV infection causes tissue ACE2 down-regulation. Higher circulating Ang II levels and less tissue ACE2 receptors leads to a systemic RAS imbalance (Tseng et al., 2020).

Possible/promising RAS treatment for COVID-19

RAS treatment via vitamine D

With the help of UV-radiation or sunlight, vitamin D, a fat-soluble vitamin and hormone is produced in the body. The active form of vitamin D is synthesized in the kidney and the liver

converts vitamin D to the principal circulating vitamin D metabolite (Gupta et al., 2021). Inadequate vitamin D levels can be linked to higher infection rates, including the coronavirus. The number of COVID-19 infections and deaths can therefore be reduced by adequate vitamin D levels (Gupta et al., 2021). This does vitamin D via different mechanisms: strengthening cellular immunity, reduction of cytokine storm with impact on interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α), maintenance of cell junctions and also the adaptive immunity is modulated by promoting T regulatory cells induction and suppressing T helper cell type 1 (Gupta et al., 2021). A cytokine storm drives persistent and excessive inflammation, which may be lethal in subjects with SARS-CoV-2 considerably. The lung vitamin D receptor is highly expressed in the lungs, making it possible treatment for COVID-19 (Gupta et al., 2021). Lipopolysaccharide-induced acute lung injury (LPS-induced ALI) is attenuated by vitamin D which modulates the RAS, renin expression and generation is inhibited (Gupta et al., 2021). Vitamin D concentration normalization lowers RAS activity by transcriptional suppression of renin expression (Gupta et al., 2021). Vitamin D also suppresses Ang II, ACE and renin expression and increases ACE2 concentration in LPS-induced ALI, the ACE2/Ang-(1-7)/MasR axis is induced and the ACE/Ang II/AT1R axis is suppressed by suppressing renin (Gupta et al., 2021). Calcitriol targets the vitamin D receptors so is an agonist of these receptors and therefore has the same effect as vitamin D on the receptors (Gupta et al., 2021). Calcitriol suppresses ACE and Ang II receptor type (AT1) and also reduces Ang II formation in hypertensive rats; the agonist also has noticeable influence on the ACE2/Ang-(1-7)/MasR axis since the expression of ACE2, MasR and Ang-(1-7) is induced (Gupta et al., 2021). A relationship between the number of COVID-19 cases and especially mortality caused by COVID-19 and vitamin D levels is observed, patients with a low vitamin D level have a higher chance of mortality (Gupta et al., 2021). Vitamin D2/D3 can be taken in orally and is safe to use as a drug (no toxicities) (Mahdavi 2020).

Vitamin D is not a RAS component but since the vitamin indirectly modulates the RAS in a positive way by suppressing Ang II and inducing the ACE2/Ang-(1-7)/MasR axis. Vitamin D can be used as a supplement against pulmonary fibrosis.

RAS blockers

ACE inhibitors that inhibit the damaging ACE/Ang II arm of the RAS in the lungs are also promising COVID-19 treatment. First, RAS blockers were used to treat cardiovascular diseases to prevent failure of heart/kidney but since ACE2 is identified as the receptor of SARS-CoV-2, RAS blockers like ACEis and ARBs gained more attention as treatment (Zhang et al., 2020). ACEis and ARBs increase the expression and activity of ACE2 but also prevent progression of pulmonary complications, there is conflicting evidence about this treatment, has it a negative or positive effect on COVID-19 (Zhang et al., 2020). To this point there is no scientific or clinical evidence supporting a harmful effect of ACEis/ARBs in context of the COVID-19 outbreak (Huang et al., 2020). ACEis and ARBs have a protective effect on all kinds of lung injuries, such as reduction of pulmonary hypertension, further research has to be done to see if lung pathological damage in patients is reduced by this treatment (Zhang et al., 2020). ACEis and ARBs are reasonable rather helpful than harmful to COVID-19 because there is no independent relationship between ACEis/ARBs and susceptibility to COVID-19 and between the RAS

inhibitors among the clinical progress of COVID-19 (Zhang et al., 2020). ACEis/ARBs may not be used simultaneously because of side effects (Zhang et al., 2020). RAS blockers are beneficial in conditions with RAS activation since RAS blockers block the Ang II/AT1R signaling pathway. AT1R is blocked resulting in reduction of vasoconstriction and inflammation. Furthermore, the counter-regulatory arm of the RAS system including ACE2, MasR and Ang-(1-7) is induced. Ang II cannot stimulate the AT1R so Ang II can only stimulate AT2R and can be converted to Ang-(1-7) (Zhang et al., 2020).

Captopril is an ACEi and candesartan is an ARB, so both RAS blockers. Pretreatment with captopril or candesartan prevents SARS-CoV-2 spike protein internalization into human type II pneumocytes, also a proinflammatory cytokine response induced by spike proteins is prevented (Calo et al., 2022). ACE2 and MasR in rat lungs are upregulated by 3 weeks of treatment with captopril or candesartan (Calo et al., 2022). ACEis and ARBs are protective in infection with COVID-19, research with rats confirmed that ACEis and ARBs contribute to reducing the proinflammatory cytokine release and decreasing the viral entry of the virus despite the increase in expression of ACE2 (Calo et al., 2022). An in vitro study on human epithelial bronchial cells investigated how RAS and Ang II blockers affected ACE2 expression, infection with SARS-CoV-2 showed that via Ang II binding to AT1R, mRNA and protein level of ACE2 are increased so SARS-CoV-2 cell entry is enhanced, irbesartan which is an ARB, abolishes these effects (Calo et al., 2022). The protective effect of the above-mentioned ARBs and RAS blockers are identified. ARBs have a protective role in the cell entry and the lack of worse outcome for patients that use RAS blockers is concluded (Calo et al., 2022). The above-mentioned treatments can be taken in as oral tablets (Calo et al., 2022).

Combinations of these medicines are not recommended, a combination of an ARB with an ACE-inhibitor leads to dual blockade and must be carried out under specialist supervision so kidney function, blood pressure and fluid and salt balance can be closely monitored (Renin-angiotensin-system (RAS)-acting agents).

sACE2

Soluble ACE2 is an engineered variant of the ACE2 receptor using deep mutagenesis. sACE2 binds tightly to the SARS-CoV-2 spike proteins and neutralizes viral infection, so prevention of infection (Gupta et al., 2021). sACE2 also cleaves Ang II, so when more Ang II is expressed, sACE2 will reduce the concentration level (Gupta et al., 2021). Since sACE2 is a protein, oral intake is difficult because of the unfavorable conditions of the gastrointestinal tract (proteolytic enzymes and harsh pH for example), the proteins will be broken down (Gupta et al., 2021). We need the sACE2 to get to the intestine since the sACE2 will be taken up there to get to the lungs (Gupta et al., 2021).

Oral delivery

Oral intake of sACE2 has been enabled which is a more patient-friendly route of administration than injection. sACE2 has the following interesting production and delivery aspects. Therapeutic proteins can be expressed within plant cells, the gene encoding for the protein is inserted into

the plant chloroplast, the expression of the protein of interest is bio encapsulated within the plant cells (Gupta et al., 2021). The cell walls of the plant cells are not hydrolyzed by the digestive enzymes of humans and therefore are the proteins protected from degradation in the gastrointestinal tract (Gupta et al., 2021). The intact plant cells with the sACE2 receptors encapsulated reach the intestine where the cell wands are broken down by commensal bacteria (Gupta et al., 2021). The therapeutic proteins get released and are absorbed in the gut lumen and from there transported to the lungs (Gupta et al., 2021). Storage and production of the protein of interest is made easier by this approach but the safety/tolerability and the pharmacokinetic profile of oral biol encapsulated sACE2 needs to be evaluated (Gupta et al., 2021).

sACE2 versus other RAS treatments

For both COVID-19 and IPF, higher Ang II levels (compared to healthy lung tissue) are present in lung tissue and this phenomenon plays a key role in these diseases (Gupta et al., 2021) (Tseng et al., 2020). An important role of sACE2 is the cleavage of Ang II to form Ang-(1-7), Ang-(1-7) stimulates the MasR and a protective effect is mediated against the disease. sACE2 has a protective effect and no discovered side-effects (Gupta et al., 2021). The rest of the RAS treatment discussed is not optimal for clinical treatment yet, the drugs are either organ toxic for a certain dose, have unfavorable pharmacokinetic properties or have a bad stability and therefore the drug needs to be modified which is expensive (Gupta et al., 2021). sACE2 has also a few disadvantages but by encapsulating the sACE2 in plant cells, oral intake is enabled and sACE2 is made and stored in the plant cells (Gupta et al., 2021). Soluble ACE2 is an enzyme and can therefore be made in the plant cell, by the plant cell itself (Gupta et al., 2021). The basic principle for the production of the sACE2 protein in plant cells is mentioned in: Oral delivery. The disadvantages of sACE2 mentioned in table 1 are not a problem anymore. Furthermore, oral intake of sACE2 is enabled, this is a more patient-friendly way of drug administration compared to repetitive injections. This principle of oral intake of sACE2 is explained in: Oral delivery.

Conclusion

A lot of promising treatments for COVID-19 and for IPF as well, are present. RAS treatment for the diseases is evaluated based on cons and pros. Most of the treatments still needs further research or modifications before it can be used as clinical treatment. RAS treatment definitely is the future of COVID-19 and IPF treatment. In my opinion, soluble ACE2 which can be orally administrated via plant cells is the future of treatment for COVID-19 and IPF. Soluble ACE2 is very promising according to multiple studies, it converts Ang II to Ang-(1-7) which stimulates MasR and soluble ACE2 prevents SARS-CoV-2 particles from entering host cells (Tan et al., 2017) (Gupta et al., 2021). A huge disadvantage for the patients was that oral delivery was not possible. Now, oral delivery is enabled by encapsulation of soluble ACE2 in plant cells; this in my opinion is the most promising treatment for both COVID-19 and IPF.

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