REVIEW

Department of Medicinal Chemistry, University of Vienna; Austria

# The role of ACE2 in the renin-angiotensin-system: Etiology and therapy of COVID-19 from a pharmaceutical perspective

C. R. Noe*, M. Noe-Letschnig

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**Corresponding author: Univ.-Prof. Dr. Christian R. Noe, Department of Medicinal Chemistry, University of Vienna, Pharmaziezentrum, Althanstr. 14, A-1090 Vienna, Austria, christian.noe@univie.ac.at*

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Angiotensin-2 converting enzyme (ACE2), a key element of the renin-angiotensin-system (RAS), is not only the direct target of infection by the human SARS-Cov-2 virus but is at the same the root for the complex pathogenetic events of COVID-19. From a pharmaceutical perspective, several established classes of medicines are involved in different phases of the disease. From their known mechanisms of action, a comprehensive understanding of COVID-19 will be hopefully soon delineated. A set of proven medicines is available to cope at least with some of the pathologies involved. To arrive back to normal life, vaccinations and broad consideration of hygienic measures are to be complemented by effective medicines to treat airborne viral infections. Therapeutic schemes based on a comprehensive understanding of the disease will include drug combinations made up from both established drugs as well as novel drugs presently under development.

## 1. Physiology and pathology of RAAS and its relevance in COVID-19

### *1.1. The Renin-Angiotensin-Aldosterone-System (RAAS)*

Angiotensin converting enzyme 2 (ACE2) was discovered only 20 years ago (Donoghue et al. 2000). Its central role as key molecule in the renin-angiotensin-aldosterone system (RAAS) was fully established around 8 years ago (Tikellis and Thomas 2012). Since then, rapidly increasing knowledge on ACE2 and the following ACE2/Ang (1–7)/Mas axis has been integrated into the known physiological and pathophysiological schemes of the RAAS (Fountain and Lappin 2020).

The peptide hormone angiotensin II (Ang II) is the main effector of the renin-angiotensin-system (RAS, as one arm of the RAAS). Generated from its precursor angiotensin I via action of the angiotensin converting enzyme (ACE) it leads to vasoconstriction. At the same time aldosterone, the effector of the second arm of the system, increases the blood volume. As a result of both, blood pressure increases. By this mechanism, the RAAS also secures liquid homeostasis of the body in general (Crowley et al. 2005).

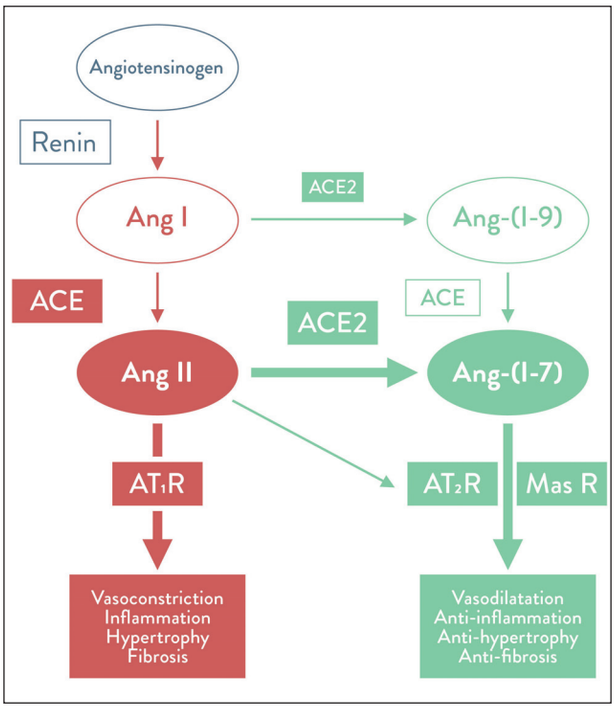
Acting directly on the vascular cell wall, Ang II and the RAS are able to direct blood to specific organs of the body with increased demand via local vasoconstriction and -dilatation, respectively (Danilczyk et al. 2004). Ang II promotes expression of several tight junction proteins (Wosik et al. 2007; Takashina et al. 2020) and growth of vascular smooth muscle cells, which is in line with its vasoconstrictor effects (Geisterfer et al. 1988; Daemen et al. 1991; Vukelic and Griendling 2014). Pathogenetic effects of Ang II include induction of pro-atherosclerotic effects (Candido et al. 2002, 2004; Cassis et al 2009), endothelial dysfunction (Loot et al. 2009), oxidative stress (Lee et al. 2019), inflammation (Chen et al. 1998) and thrombosis (Senchenkova et al. 2010). In a dysfunctional state it may eventually induce fibrosis (Geisterfer et al. 1988; Kaschina et al. 2000; De Gasparo et al. 2003; Murphy et al. 2015). The RAS represents a tightly regulated network. Dysfunction at any point may lead to common final vascular events like infarctions of heart or brain, which are the most frequent cause of human death.

Abnormal activation of the RAS and progression of atherosclerotic vascular disease are directly connected (Jacoby and Rader 2003; Johnstone 1994; Lee et al. 1993). Inhibition of Ang II has proven to be highly effective in the treatment of hypertension. Two out of five major drug classes recommended for the treatment of arterial hypertension and prevention of cardiovascular disease target the RAS: ACE inhibitors (captopril and other -prils) and ARBs (angiotensin-II-receptor antagonists: losartan and other -sartans, also named either AT-antagonists or $AT_1$ antagonists). Both, ACE inhibitors as well as ARBs are among the most widely used classes of antihypertensive drugs. They have similar and high effectiveness regarding major cardiovascular events and mortality outcomes. ARBs are associated with significantly lower treatment discontinuation rates for adverse events than those of all other antihypertensive therapies (ESC/ESH 2018). Over the past 40 years they probably were – among all medicines – the therapeutic group contributing most to prolongation of human life expectancy.

### *1.2. ACE2*

After discovery of ACE two decades passed until the physiological counterpart ACE2 was discovered as a further key element of the RAS. ACE2 downregulates the activity of Ang II by cleaving it to Ang (1-7), resulting in reduction of the blood pressure (Turner and Hooper 2002; Rice et al. 2004). Effects of the degradation product Ang (1–7) oppose those of Ang II in the vasculature (Probstfield et al. 2010; Ferraio et al. 2005). Ang (1–7) shows anti-inflammatory and antioxidant properties (Probstfield et al. 2010; Ferraio et al. 2005). The interplay between the two enzymes ACE – generating Ang II – and ACE2 – removing it by cleavage – is of fundamental importance. Increased blood pressure induces expression of ACE2 as counter-regulation (Patel et al. 2014; Xia and Lazartigues 2010). Consistently, lack of ACE2 results in physiological and pathophysiological responses of increased levels of angiotensin II (Ashraf et al. 2020). Their organ-specific activity in vascular membranes secures homeostasis of blood pressure as well as fluid and electrolyte homeostasis. The system controls the blood flow in tissues and organs according to requirements (Patel et al. 2017).

ACE2 is differentially expressed in a variety of organs and tissues. Recent results on expression patterns contradict earlier findings (Tipnis et al. 2000; Hamming et al 2004) with respect to the respiratory system and showed respiratory epithelia to be negative for ACE2 (Matusiak and Schürch 2020; Hikmet et al 2020; Zou et al. 2020). The data include expression of viral entry co-factors




Scheme 1: Enzymatic angiotensin processing: ACE = angiotensin converting enzyme, Ang = angiotensin, $AT_1R$ = angiotensin receptor subtype 1, $AT_2R$ = angiotensin receptor subtype 2, Mas R = mitochondrial assembly receptor

transmembrane serine protease 2 (TMPRSS2), basiginin (BSG, CD147) and furin (Matusiak and Schürch 2020) and infection risk for specific organs (Zou et al. 2020). The fact that ACE2 is upregulated in the airways of smokers has prompted discussion on the effect of smoking on COVID-19 infection (Smith et al. 2020; Matusiak and Schürch 2020). In view of the role of the RAS in regulating blood flow via vascular constriction and dilatation, local and time dependent differences in expression dynamics regarding different organs are to be expected anyway (Roca-Ho et al. 2017) to allow adaption of blood pressure via liquid and electrolyte supply. It may be hypothesized that the potential for immediate efficient ACE2 expression in vascular membranes is essential for vital organs, such as lung, kidney, heart, and brain, which have a requirement for stringently controlled supply. This is in line with the fact that in patients with type 2 diabetes and kidney disease a decreased expression of ACE2 was observed (Tikellis et al. 2003; Reich et al. 2008; Soler et al. 2008; Roca-Ho et al. 2017; Matusiak and Schürch 2020).

### *1.3. ACE2 and COVID-19 infection*

With respect to COVID-19 infection, epithelial and endothelial expression of ACE2 is of eminent importance. The fact that ACE2 is the point of attachment of the spike protein of the SARS-Cov-2 virus (Zhou et al. 2020; Hoffmann et al. 2020; Li et al. 2003) has promoted the enzyme to enormous public attention. During the first phase of the pandemic in Lombardy it was observed that a significant share of the hospitalized patients was treated with antihypertensive drugs. The correlation between antihypertensive medication and COVID-19 infection was recognized and followed up (Mancia et al. 2020; Shukla and Banerjee 2021). In COVID-19the ACE-ACE2 equilibrium is shifted to reduced ACE2 activity and elevated Ang II levels. Compensation by increased ACE2 expression would be a normal physiological process, but lead again to an increased opportunity for viral attack. This argument, used in the discussion on therapy of COVID-19 with ACE blockers and ARBs, was not confirmed (Sriram and Insel 2020). Neither ACE inhibitors nor ARBs have a negative effect on the course of a COVID-19 infection (Mancia et al. 2020; Ramos 2020). Efforts of researchers and clinicians on the use of these drugs are going on – including studies on genetic effects (Bosso et al. 2020 et ref. cited) – to resolve the partly contradictory observations on positive effects of treatment of Covid-patients with AT-antagonists and ACE-inhibitors.

Severe cases of COVID-19 evolve into severe acute respiratory syndrome (SARS) with hyperinflammation (cytokine storm), cellular invasion, massive lung edema, and finally fibrosis. Effects like inflammation and fibrosis are related to increased Ang II activity. There is almost self-explanatory evidence that the attack of the virus on ACE2 goes beyond an infectious process and will impair the function of the RAS in general (Abassi et al. 2020). The specific tragedy and threat of the Corona pandemic is given by the fact that the virus interferes directly with ACE2, the important effector and gatekeeper of the RAS, with a potential of heavily disturbing the equilibrium of the system. The impressive life prolonging progress in medication of hypertensive disorders achieved over the last decades is challenged by SARS-Cov-2.

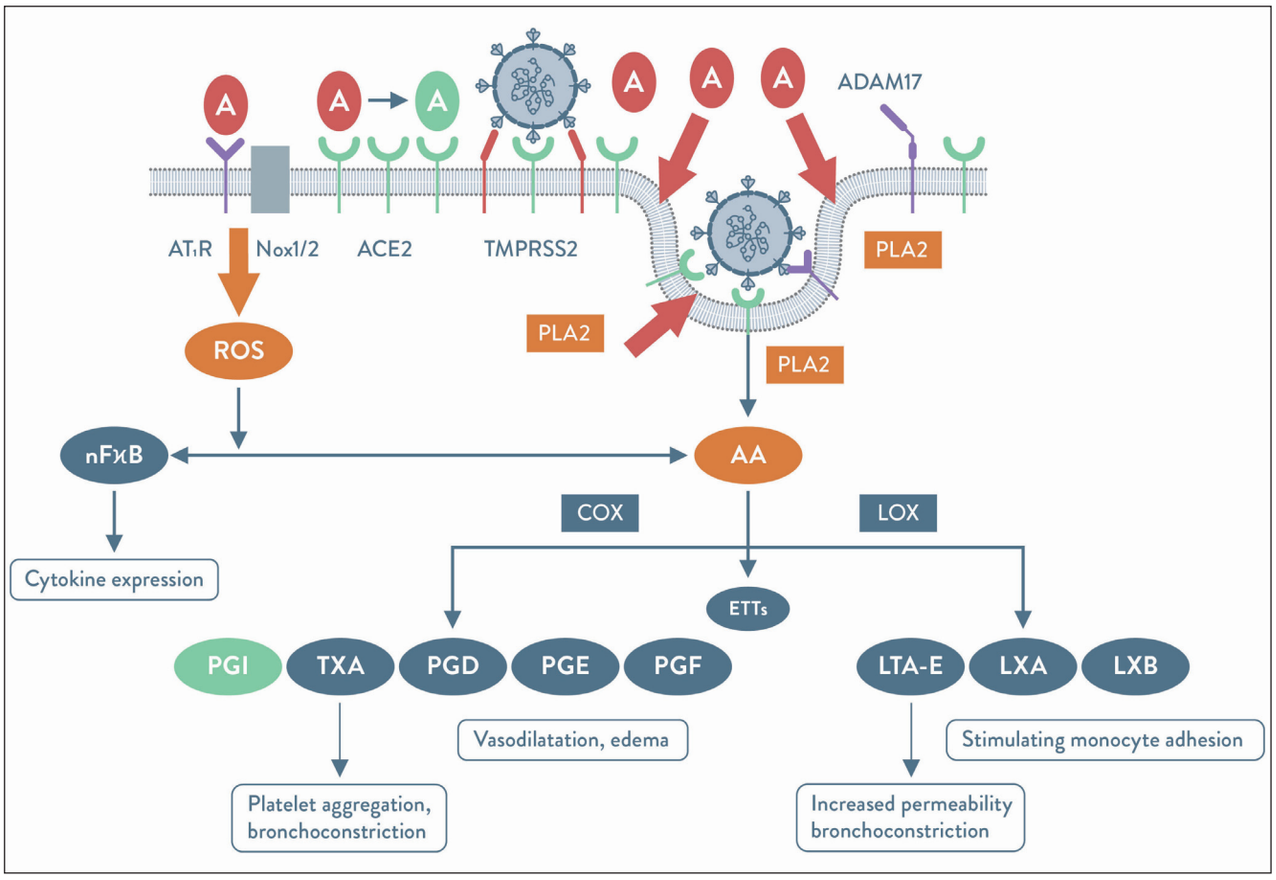
### *1.4. The metabolic syndrome and COVID-19*

The risk to suffer or even die from a COVID-19 infection is directly correlated to age, metabolic disorders and diseases. Legal and regulatory measures take this into account, which is correct from a statistical point of view. The fact that even young people die from Covid causes irritation and is perceived as outlier or as consequence of mutations. Frequently, a severe progress of the disease is found in patients with high blood pressure, chronic heart and kidney diseases, diabetes, and obesity (Wu and McGoogan 2020; Huang et al. 2020; Wang et al. 2020). The majority of these disorders is part of the metabolic syndrome. Around half of the adult population of the United States is suffering from hypertension, with the percentage increasing with age (CDC 2019). The situation in other industrialized countries is similar. Atherosclerosis has been recognized to be another important pathogenetic factor arising from a metabolic syndrome (Mathieu et al. 2006). At the same time, it has been shown that ACE2 expression is reduced in established atherosclerotic plaques (Sluimer et al. 2008). As mentioned above, hypertension and its therapy link the metabolic syndrome directly to COVID-19. This correlation supports the hypothesis that SARS Cov-2 – due to its docking point ACE 2 – may interfere broadly with the RAS in the course of the disease. The correlation between the metabolic syndrome and COVID-19 allows a data based, age independent definition of a high-risk patient along those diagnostic criteria, which are well established for the metabolic syndrome. Regardless of the metabolic syndrome, vascular function decreases with age, in general. Also, this is in line with the age-related infection risk with COVID-19.

### *1.5. Interaction of the RAS with the AA cascade and its relevance in COVID-19*

Any entry of a pathogen proceeds by attachment to a membrane *via* adhesion molecules, expressed on both interacting partners. By interference with the cellular membrane, an infection will generally activate the arachidonic acid (AA) cascade (Hoxha 2020) and induce inflammation. This mechanism takes also place in the case of COVID-19, thus placing the AA system into the etiology of COVID-19 (Ripon et al. 2021).

With SARS-Cov-2 infection, interaction between the RAS and the AA-cascade obtains an additional severe momentum. As mentioned before, the main adhesion molecule of SARS-Cov-2 is the spike protein. Its counterpart is membranous ACE 2. Attachment of the virus at the apical side of endothelial cell membranes will disturb their integrity in any case. It may be hypothesized – but has not yet been shown – that membrane inflammation, whether preexistent or induced, will additionally support virus adhesion and progression of infection, as inflammation in general induces expression of adhesion molecules (Meager 1999). In any case, two important physiological systems, the RAS and the AA-cascade, converge into a joint pathophysiological process. Disturbed membrane integrity will activate the inflammatory AA-system. At the same time, the




Scheme 2: The arachidonic acid system in SARS-Cov-2 infection: A (in red) = angiotensin 2, A (in green) = angiotensin-(1-7), ACE2 = angiotensin converting enzyme 2, $AT_1R$ = angiotensin receptor subtype 1, TMPRSS2 = transmembrane serine protease 2, PLA2 = phosopholipase 2 (phosphatidylcholin-2-acylhydrase), Nox1/2 = nicotinamide adenine dinucleotide phosphate oxidase isoforms 1 and 2, ROS = radical oxygen species, ADAM17 = a disintegrin and metalloproteinase domain 17, AA = arachidonic acid, COX = cyclooxygenase, LOX = lipoxygenase, ETTs = epoxyeicosatrienoic acids, PG = prostaglandin, TXA = thromboxane A, PGI = prostacyclin (prostaglandin I), LTA-E = leukotrienes A-E, LX = lipoxin

complex functional equilibrium of ACE and ACE2 (Gaddam et al. 2014) will become increasingly dysfunctional. Reduced activity of membranous ACE2 will result in increased local blood levels of circulating Ang II, promoting direct inflammatory, thrombogenic and fibrinogenic effects in addition to vasoconstriction. Reduced functional activity of ACE2 in membranes of blood vessels in other organs than the lung may lead to a local excess of Ang II in patients suffering from endothelial inflammation. This explains disease symptoms and infections of vulnerable people in organs beyond the lung and holds also true with brain vessels, which constitute the blood-brain-barrier (BBB), supporting inflammatory and thrombogenic processes and opening the path for invasion of the virus into the brain (Reynolds et al. 2021).

It is known that Ang II directly activates phospholipase A2 (PLA2), the enzyme which releases AA from the membrane (Jacobs and Douglas 1996; Khan et al. 2016). It is also known that Ang II activates membranous NADPH oxidase (Nox) (Li and Shaw 2003; Nguen Dinh Cat et al. 2013; Drummond and Sobey 2014; Konior et al. 2014; Zimnol 2017; Violi et al. 2020) either directly or via the $AT_1R$ (Pendergrass et al. 2009), generating reactive oxygen species (ROS) (Fernandes et al. 2020), which will react with AA to induce the AA-cascade. It seems that known pathophysiological mechanisms related to COVID-19 – such as endothelial dysfunction, oxidative stress, inflammation or thrombosis – are a result of the overlapping of the RAS and AA systems (Jin et al. 2020). Aggravation of inflammation due to the described interaction of these two systems may be expected to boost stimulation of the LOX branch of the AA system, causing severe symptoms of inflammation including the capillary leak syndrome (Funk and Ardakani 2020). This hypothesis is supported by the remarkable therapeutic results achieved with the leukotriene-antagonist montelukast in severe cases of COVID-19 (Aigner er al. 2020; Khan et al. 2021). The key molecule in the upstream cascade of inflammation is the transcription factor nF-κB, which triggers expression of a plethora of gene products, mainly inflammatory cytokines, chemokines and adhesion molecules. Ang II is a known activator of nF-κB (Kim et al. 2020). This activation proceeds *via* the ROS pathway. The cytokine storm, typical for severe forms of COVID-19, may be considered as a potentiating interplay of the RAS and the AA cascade.

### *1.6. Long Covid*

After more than one year since the onset of the pandemic, an increasing number of patients is observed suffering from ongoing symptoms of the Covid disease in a variety of organs, called Long Covid. In view of the central role of ACE2 for maintenance of the blood pressure in the whole body it is not at all surprising that this point of attack is equally offered to the virus in many organs. Persisting inflammation in other organs than the lung, such as the nervous system, may lead to severe and long-term side effects of a COVID-19 infection as seen in Long Covid (Wu et al. 2020; Jacobs 2021).

Endothelial inflammation will at the same time induce expression of adhesion molecules thus provoking microbial infection, in a process strongly resembling thrombocyte action. The adhesion step prepares the entry of the microorganism into the cell. Prostaglandin produced by an inflamed membrane will attract microorganisms, which will attach to the inflamed tissue via adhesion molecules. In many cases, microorganisms themselves are in a position to produce prostaglandins to induce the initial step of

tissue inflammation, resulting in a self-sustaining cycle of chronic inflammation-infection (Noe-Letschnig 2020). In connection with a dysfunction of the BBB this process has been linked to neurodegeneration (Noe et al. 2020). Persisting SARS-Cov-2 virus might also take part in a chronic vascular infection-inflammation process.

## 2. Prevention and therapy of COVID-19

### *2.1. Diagnosis*

A COVID-19 infection diagnosis starts with a positive PCR test, at least as far as public awareness is considered. From the patient's perspective, "infection" has a very broad meaning. Until the beginning of the pandemic "infection" was clearly distinguished from "colonization" and was defined by the concomitant presence of an infectious agent and related symptoms. In 2020, the term "Corona positive" covers conditions from the mere presence of RNA particles and inactivated virus in mouth, nose and throat, to presence of active virus under control of the immune system without symptoms, to mild infection with symptoms resembling at least partly to common cold, to severe infection requiring hospitalization and finally to emergency treatment, a stage in which dysfunction of RAS due to the impact of infection creates a life threatening situation.

### *2.2. Vaccinations*

Rapid development of SARS-Cov-2 vaccines, fast track registration, global distribution and large-scale vaccinations of whole populations is without any doubt one of the most impressive success stories in the global history of pharmaceutical industry. Not surprisingly, vaccination against SARS Cov-2 is a topic of public discussion in several aspects, one of them being increasing incidence of rare severe side effects. In general, clinical projects on vaccinations are long term enterprises. With observed fatalities, critical consideration is required, because it is doubtful, whether those healthy young women who died from vaccination against SARS-Cov-2 would also have died from COVID-19. Remarkably, the most severe adverse reactions observed have been lethal brain vein thromboses and cases of capillary leak syndrome, both being otherwise extremely rare conditions. These thromboses have been related to immunological effects, similar to heparin induced thrombosis (McGonagle et al. 2021 3(3); McGonagle et al. 2021 3(5); Greinacher et al. 2021; RKI 2021) In general, thrombocyte aggregation is also linked to Ang II activity and thromboses are a typical side event in a disturbed RAS (Senchenkova et al. 2010; Abassi et al. 2020). It is known that the highly regulated RAS and blood coagulation may be easily disturbed by seemingly minor changes in lifestyle: Pregnancy, the use of contraceptives, smoking or obesity increase the risk of thrombosis (Cushman 2007). In view of the well-known thrombotic complications in COVID-19 infection, the potential for thrombotic adverse events with the spike protein as vaccination target should not be excluded. An antibody against the spike protein will at least exhibit partial structural similarity with the binding domain of Ang II at ACE2. For this reason, it may be hypothesized that such antibodies might bind to Ang II preventing its degradation to Ang (1-7). Especially long-term physiological consequences of such circulating complexes and a concomitant disbalance of the RAS are completely unknown. Capillary leak syndromes could be due to inhibition of expression of tight junction proteins or could be the result of an AA cascade induction via the LOX pathway (Funk and Ardakani 2020). Also, this might be induced by persisting Ang II, its complexes with antibodies, or the deficiency of Ang (1-7).

Heavily disputed from the beginning, further evidence has been presented that reverse-transcribed SARS-CoV-2 RNA can integrate into the genome of cultured human cells and can be expressed in patient-derived tissues (Zhang et al. 2021). It is yet unclear, whether this observation is of clinical relevance, whether it is related to virus RNA alone or might also take place with mRNA vaccinations. Although there is low probability that this is the case or that this might play a role in Long Covid, careful assessment of long-term side effects is mandatory, last not least to support the new technology of mRNA vaccinations.

All vaccines presently used in Europe are targeting the spike protein of the virus (Dai and Goa 2021). Knowledge on structural details is required to correlate variations in their binding domain to adverse effects of vaccinations. Comparative studies on rare adverse effects of all vaccines in use, guided by regulatory agencies, might contribute to a clarification, and help to cope with potentially occurring side effects. This is important in view of the vaccination of the whole population, starting with infants.

### *2.3. Prevention*

Disease prevention *via* hygienic measures is the first priority in handling the Corona disease and other viral pandemics. These measures comprise hand hygiene, oral hygiene, and air hygiene. At present, periodontitis induced by SARS-Cov-2 is one of the most frequent side effects of COVID-19 disease (Marouf et al. 2021). Tooth cleaning, gargle and anti-inflammatory lozenges are very effective measures of oral hygiene to reduce viral load in mouth and throat thus reducing the risk of infection progression. Although pointed out by experts (e.g. Noe and Sprinzl 2021), public awareness of oral hygiene is too low due to a lack of effective communication. Wearing masks has proven its efficacy by reducing infection and is the most effective measure to prevent direct one to one infection. Recent data confirm that aerosol infection plays an important role in COVID-19 infection (Tang et al. 2021). Depending on the degree of activity, humans exhale approximately 500 L of air and even more within one hour. SARS-CoV-2 levels in exhaled breath of an infected person can reach 100.000 to 10.000.000 copies per cubic meter, if an average breathing rate of 12 L/min is assumed (Ma et al. 2019). A significant part of this will remain in the air as aerosol. Thus, within short time a small unventilated room may become a super-spreading environment for people staying there in the presence of an infected person. Small infectious aerosol particles in unventilated rooms remaining in the air for a long time are the source of direct lower airway infection (Tang et al. 2021). Creating awareness for air hygiene and control of air quality (Hobday and Dancer 2013; Kampf et al. 2020; Morawska et al. 2020) are indispensable steps to avoid general lockdowns in the future.

Upper and lower respiratory tract infections must be considered as separated steps of infection, the latter triggering severe cases of the disease (Madas et al. 2020). Upper airway SARS-CoV-2 infections will show symptoms partly comparable to other viral airway infections, such as common cold and flu. Although there is at present no proven medication for treatment, the immediate application of existing homespun remedies and OTC medicines is advisable, last not least to reduce the viral load and to avoid shedding into lower airways. Inhalation of anti-inflammatory or antiseptic terpenoid vapors is also suited to prevent infection or to provide relief at least (e.g. Eckes 2020). Such preventive actions, in a manner similar to viral infections in general, have not been considered sufficiently with COVID-19. Remarkably, due to the broad scope of anti-Covid measures, flu medications were one of the product groups in pharmacies with reduced turn-over during winter 2020 (Porter 2021).

### *2.4. Medical therapy of COVID-19 infection*

Regarding pharmaceutical sciences and practice, the situation concerning COVID-19 therapy is at least disappointing. As outlined above, the etiology of the disease is complex and related to several pathophysiological pathways. Therapeutic measures are applicable and necessary for prevention, for upper respiratory tract infection (Lovato et al. 2019; Madas et al. 2020), for acinar airway infection (Madas et al. 2020), for systemic infection (Temgoua et al. 2019), for inflammation (Hoxha 2020), for acute respiratory distress syndrome (ARDS) (Khan et al. 2021), for severe reactions of the immune system peaking in a cytokine storm (Mishra et al 2019, Garcia 2020), for fibrosis (Zou et al, 2021) and for Long Covid, a series of follow-up diseases related to several organs

(Temgoua et al. 2019; Dennis et al. 2021). In spite of this complex situation, a set of established medicines is available to support the treatment of many of these pathologies immediately. A short survey on available options will be given in this chapter.

With the Corona pandemic, existing regulations of WHO and regulatory agencies, which are foreseen in the case of a pandemic, had a detrimental effect. Promised advantages in regulation and protection in markets under "first come-first serve" conditions prompted a highly competitive race among companies and institutions aiming to be the privileged first in the market. Unfortunately, the conditions of this race were not specified enough. Instead of classifying therapeutic options within categories as mentioned above, everything was put into the basket "COVID-19 infection". Antivirals were competing with antimalarials and immunomodulators. From a huge global effort, a large variety of potentially useful registered drugs appeared, complemented by drug candidates derived from earlier R&D projects and SARS-Cov-2 screening of compound libraries. Taking all available medications together (Chilamakuri and Agarwal 2021), they are still far from providing a comprehensive therapeutic solution for COVID-19. In the meantime, WHO is listing a series of "myth-busters" that have been popping up as alleged final solution for the pandemic and disappearing as quickly (WHO).

### *2.4.1. Anti-viral therapy*

Remdesivir made the race and seemed to be the solution at least for a moment (Beigel et al. 2020), until WHO declared it ineffective (Dyer 2020). Despite its limited efficacy, remdesivir is of use in early stages of the infection and is also frequently used in combination regimes in the progressing disease. After the hype and the following disappointment regarding this drug, political attention switched over to testing strategies and vaccinations. Since then, comparably little attention and support has been attributed to the development of effective medicines against COVID-19. In view of continuous mutations of SARS-Cov-2 and the fact that there is no effective therapy against any airborne viral infection at all, this seems to be rather short-sighted. Despite limited public interest, numerous projects searching for antiviral drugs are on the way, several of them repurposing projects, others starting out from compound libraries screening or aiming at new targets (Frediansyah et al. 2021; Adamson et al. 2021; Krumm et al. 2021). In any case, timelines for small molecule drugs addressing novel targets will not provide short-term success due to the complex process of clinical drug development. Screening of registered drugs for use in COVID-19 has provided a few results, such as favipiravir (Dabbous et al. 2021; Joshia et al. 2021). Several other candidates, among them molnupiravir (Painter et al. 2021) are in development. Most therapeutic efforts for antiviral therapy target RNA replication. Inhibition of protein processing, in particular of the SARS-CoV-2 proteases PLpro and Mpro, add to this (Luan et al. 2020; Gil-Moles et al. 2020; Kouznetsova et al. 2020, Krumm et al. 2021). Host based targets include ACE2, TMPRSS2, furin, cathepsin L and several kinases (Gil-Moles at al. 2020). Some development candidates, such as genistein, resveratrol or raloxifene, aim at inhibition of protein expression (Hong et al. 2021; Pasquerau et al. 2021; Salman et al. 2020). It may be expected that combinations of drugs addressing different viral targets might soon become a promising approach. Among the projects related to biologicals, therapeutic application of soluble recombinant ACE2 is particularly interesting and has progressed far in clinical development (Apeiron 2021). In general, it has to be stated, that so far the efficacy of investigated anti-viral substances was limited to early phases of COVID-19. Additional draw-backs are related to the systemic use of compounds, which hampers broad application in out-patient settings as a preventive action. Most antiviral drug candidates do not address the early infection step itself. Projects related to this should go along with general measures for infection prevention.

### *2.4.2. Supportive medication*

At this moment, medical treatment of COVID-19 is limited to supportive measure in cases showing rapid deterioration up to life threatening conditions. Therapy is then focused mainly on providing oxygen, on attenuation of an exuberant immunological response and prevention of thrombosis. Anti-thrombotic drugs (Godino et al. 2021; Maldonado et al. 2020; Vivas et al. 2020) and glucocorticoids (Solinas et al. 2020; Ferrara and Vitiello 2021) are mostly used. Oxidative stress (Suhail et al. 2020) and dysregulation of Nox-dependent signaling have been identified as a potential mechanism to target severe COVID-19 (Damiano et al. 2020). Dexamethasone, the first drug proven to reduce mortality (Recovery Collaborative Group 2021) and glucocorticoids in general are very effective to suppress immune reactions in late stages of a severe course of the disease. However, it remains questionable, whether an off-label use of budesonide in early phases of the disease (Ramakrishnan et al. 2021) or even in prevention, makes sense. The known suppression of PLA2 action by glucocorticoids (Bailey 1991) prevents the inflammatory response but renders patients even more susceptible against infection (Ellepola and Samanaranayake 2001; Robinson and Morand 2021; Youssefcet al. 2016).

The management of the cytokine storm is a particular challenge in Covid emergency treatment. To cope with an exaggerated production of proinflammatory cytokines, the recruitment of proinflammatory macrophages and granulocytes (Tufan et al. 2020) an array of biologicals developed and commonly prescribed to individuals with autoimmune and inflammatory rheumatic diseases is available (González-Gay et al. 2021). The interleukin (IL)-6 antagonists tocilizumab and sarilumab (Crisafulli et al. 2020), the IL-1 antagonist anakinra (Cron 2021), the JAK inhibitor baricitinib (Goletti and Cantini 2021), the anti-complement C5a monoclonal antibody eculizumab (Diurno et al. 2020) and anti-tumor necrosis factor (TNF)-alpha agents are the most frequently used among them. Convalescent plasma therapy adds to this (De Santis et al. 2021).

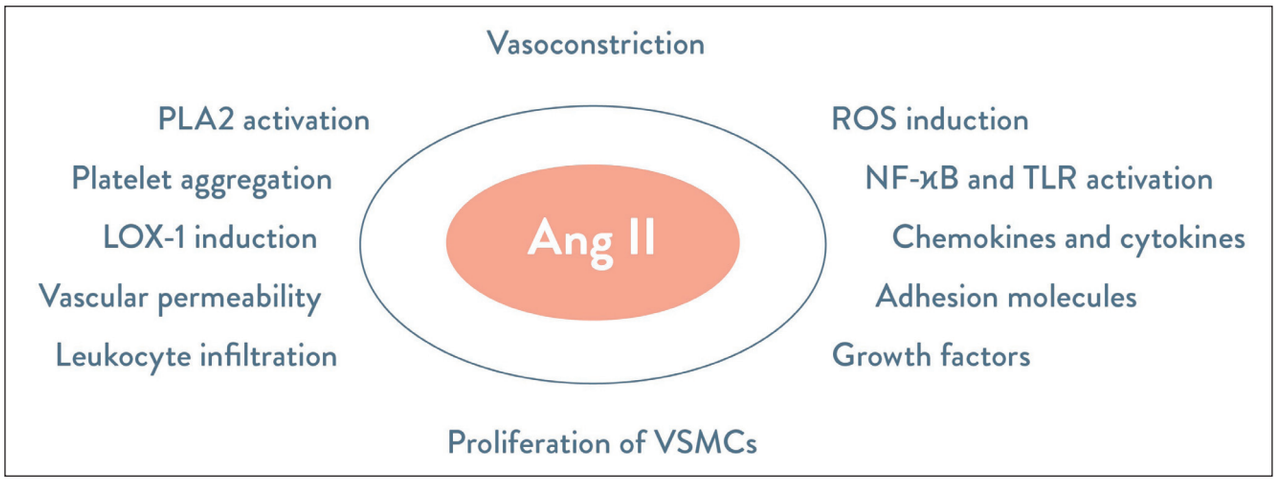
### *2.4.3. Outlook: Systemic approaches to address a complex etiology*

There is an ongoing shift of strategy in drug research away from single target approaches, in which the goal is to obtain an API perfectly adjusted to a (preferably new) target, to systemic approaches, in which the goal is to intervene with the dysfunction of a physiologic system, such as endothelial dysfunction going along with COVID-19, which has been already recognized as such a systemic target (Jin et al. 2020).

In complex diseases a systemic approach by combinations of APIs is generally superior to compounds targeting only one position in the network. A new medicine may also be a combination of established APIs. This provides faster and cost efficient clinical development due to a highly reduced safety risk, as well as proven efficacy at the selected targets. Both the complex etiology of COVID-19 and the fact that there is an existing set of registered APIs ready for use in humans renders a systemic approach almost mandatory. However, this does not mean to simply mix APIs, but requires careful combination and observation of the results.

Etiology and progression of a COVID-19 infection seem to be dominated by the joint action of the dysfunctional RAS with the AA-system. Therefore, in addition to anti-infectives (antivirals), the application of the following three distinct classes of existing therapeutics – including their combinations – comes into play: anti-inflammatory drugs (NSAIDs, LOX-inhibitors), immunomodulators (glucocorticoids, biologicals) and antihypertensive drugs (ACE inhibitors and ARBs). Based on an improved understanding of the etiology of the disease in all its phases, medicines can be designed swiftly from the existing set of APIs to be complemented and further optimized by new compounds. A few considerations on this are given in the following:

With respect to inflammation, in addition to glucocorticoids, also NSAIDs – e.g. ibuprofen (Smart et al. 2020) – have proven to be




Scheme 3: Effects of angiotensin II: Ang II = angiotensin II, LOX-1 = lipoxygenase 1, PAI-1 = Plasminogen activator inhibitor-1, PLA2 = Phospholipase A2, NFkB = nuclear factor 'kappa-light-chain-enhancer' of activated B-cells, TLR = toll-like-receptor, VSCM = vascular smooth muscle cell

safe (Drake et al. 2021). They reduce inflammation, relieve pain, and prevent thrombocyte aggregation. They also might be helpful by preventing the progression of the infection in early stages of the disease due to their ability to suppress endothelial cell adhesion molecules, which are elevated in COVID-19 Patients (Tong et al. 2020; Li et al. 2021). Cellular adhesion is also promoted by the Von Willebrand factor (Dejana et al. 1989). In severe cases of COVID-19 disease, acute and sustained endothelial cell activation by the propeptide of this factor has been observed (Ward et al. 2021), a finding that opens up additional therapeutic options (Gragnano et al. 2017). As mentioned above, the LOX-inhibitor montelukast was shown to be effective in the treatment of severe cases of COVID-19 (Aigner et al. 2020).

The use of antihypertensive drugs (ACE inhibitors and ARBs) has been already discussed above (Mancia et al. 2020; Shukla and Banerjee 2021). The fact that there is a direct link between Ang II, the main driver of COVID-19, and the symptoms of severe course of the Covid disease deserves particular attention. Combinations of antihypertensive drugs with anti-inflammatory drugs may provide options for treatment and prevention of a lethal outcome of a COVID-19 infection.

The application of gold compounds in COVID-19 therapy (Gil-Moles et al. 2020; He et al. 2020; Rothan et al. 2020) also seems to be a promising approach. Gold is a very unreactive element, with exception of its high affinity to sulfur atoms. These are found widely distributed in the body, preferably in cysteine-rich domains of reactive proteins. In addition, gold ions are strong cations, able to replace other metals in catalytic positions of enzymes (Gil-Moles et al. 2020). As a result of these effects, gold drugs (e.g. aurothioglucose, aurothiomalate and auranofin) selectively exert attenuating physiological effects at specific targets in the body. With nF-κB being their most preferred target, the traditional use of gold drugs as antirheumatic medicines is mechanistically justified (Williams et al. 1992). Their reported antiviral and anti-bacterial activity (Okada et al. 1993; Elkashif and Seleem 2020) create a unique pharmacological profile for this class of compounds. Recently it has been shown that gold compounds also specifically target the spike protein of SARS-Cov-2 and other functional components of this virus (Gil-Moles et al. 2020; He et al. 2020; Rothan et al. 2020). Since gold compounds address equally infection, inflammation and immunomodulation, the three dominant systems of COVID-19 pathology, they might provide promising therapeutic options.

Extensive research efforts contribute almost daily to an improved understanding of the COVID-19 disease. The number of more than 4000 clinical studies related to COVID-19, listed by WHO, is proof of the impressive global activity of the research community to fight the pandemic (WHO 2021). The existing and fast developing pool of compounds active against the different pathological aspects of COVID-19 will provide opportunities to develop efficient combination therapies if based on a coherent therapeutic concept.

## 3. Conclusions

Forever, the pharmaceutical task has been to apply the best available medication to patients, to understand the underlying pharmacological mechanisms and to improve the "treasure of medicines". With COVID-19, several physiological systems of high relevance in pharmacotherapy merge into one disease. ACE2, the immediate target of the infection, is at the same the root for the pathogenetic events resulting in severe forms of COVID-19. Ang II is the main driver towards severe stages. Not surprisingly, the number of existing drugs interfering with one or the other of the physiological systems involved is rather high, in principle. Nevertheless, therapeutic success will remain limited, unless the multifactorial character of the COVID-19 disease is taken into account. To arrive at normality in everyday life, careful attention of hygienic measures is equally important as vaccinations. The urgent need for an effective therapy of COVID-19 and similar viral diseases with potential to evolve into a pandemic can build upon broad theoretical knowledge and safe medicines but requires further concerted efforts.

Conflict of interest: CRN and MNL are both involved in projects related to prevention and therapy of COVID-19, but do not see a conflict of interest.

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