Dance Round COVID-19

WE DANCE ROUND IN A RING AND SUPPOSE,
BUT THE SECRET SITS IN THE MIDDLE AND KNOWS.
ROBERT FROST

NEW CHALLENGES TO RENIN-ANGIOTENSIN-SYSTEM IN COVID-19 PANDEMIC

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*In the fight against the global epidemic from the new corona virus (SARS-CoV-2), awareness on the site of the primary viral attack, the so-called „entry port“ enables an implies efficient prophylactic/therapeutic approach. The attack is aimed at the important balancing unit of the renin-angiotensin system (RAS), angiotensin-converting enzyme 2 (ACE2), which regulates the level of angiotensin II (Ang II). While Ang II has vasoconstrictor and inflammatory functions, the ACE2 converted product of Ang-(1-7) possesses vasodilating and anti-inflammatory functions. In patients with pathological cardiovascular symptoms and increased blood pressure, maintenance of optimal Ang II is achieved by inhibiting the synthesizing enzyme ACE1 or blocking the angiotensin receptor response (ATR). In this Dance Round, an attempt is made to address the question: In the unbalanced functions of RAS (manifesting as an outcome of SARS-CoV-2 epidemic), will the therapeutic effect of ACE1 inhibitors change and in what direction? **Biomed Rev 2020; 31: 105-112***

Keywords: angiotensin, angiotensin-converting enzyme, ACE inhibitors, SARS-CoV-2, COVID-19

INTRODUCTION

We live in a time of respiratory infection epidemic caused by a new representative of the Corona virus family – SARS-CoV-2. Scientists of diverse expertise around the world have made extraordinary efforts to obtain as much information about the virus as is possible: its nature, path of spread, and the major goal naturally, was to understand the “front door” for the viral attack to organism. That is, how does the virus enter

our body? The answer to this turned out to be a protein found in our cells, abbreviated as ACE2 (angiotensin-converting enzyme 2), which also serves as the entry portal for another coronavirus (SARS-CoV) (1). ACE2 is an important enzyme involved in the functioning of a chain of peptides, within the renin-angiotensin-system (RAS) (Fig. 1). It is very crucial to cardiac health, fluid and salt homeostasis. This issue assumes high relevance with the mass, as co-morbidities are associated

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with Coronavirus infections. Several patients routinely use inhibitors of ACE1, an enzyme with quite distinct functions from ACE2 (2). Naturally, commoners and experts alike are intrigued at what direction this therapeutic approach will change the ACE1/ACE2 balance, which is compromised in the context of viral infection (3).

A brief description of the RAS system would allow us to understand the consequences of the viral hijack of the physiological processes better. Renin cleaves a decapeptide, angiotensin I (Ang I), from renin's substrate (Angiotensinogen). Ang I has little or no biological activity and is a substrate for several plasma peptidases; one of which is ACE1, a dipeptidyl carboxypeptidase. This forms the octapeptide angiotensin II (Ang II) from angiotensin I. By activating the angiotensin receptor (AT1R), Ang II serves as the main trigger leading to vasoconstriction, fibrosis, inflammation and altered redox balance. Further, ACE2 shortens Ang II peptide to Ang-(1-7). Ang-(1-7) is an agonist at both AT2R receptors and its own

G-protein coupled receptor, Mas. That activation elicits quite distinct responses, such as vasodilatation, anti-inflammatory and anti-fibrotic activity.

At first glance, it is clear that the SARS-CoV-2 viral attack would disturb the “homeostasis” of the RAS, i.e. the balance between ACE1/Ang II/AT1R axis and ACE2/Ang-(1-7)/Mas axis.

In the present *Dance Round*, we would like to (i) describe the main players in the RAS (ACE1 and ACE2), (ii) specify their altered functions for cardiovascular and reproductive systems in the context of COVID-19 epidemic, and (iii) answer whether the effects and responses to ACE1 inhibitors (ACE-I) change under these conditions.

1. ANGIOTENSIN-I CONVERTING ENZYME, FUNCTIONS

Angiotensin-I converting enzyme (ACE, dipeptidyl carboxypeptidase I, kininase II, EC 3.4.15.1) is a membrane-bound zinc-containing multifunctional enzyme. Although

Figure 1. The renin-angiotensin system (RAS) cascade and angiotensin-converting enzyme (ACE) and angiotensin receptor 1 (AT1R) inhibitors action. Ang I: angiotensin I; Ang II: angiotensin II; ACE: angiotensin-converting enzyme; ACE2: angiotensin-converting enzyme 2; AT1R: angiotensin II receptor type 1; AT2R: angiotensin II receptor type 2; ACE-I: ACE inhibitors; AT1R-I: angiotensin receptor 1. → transformation; ⊥ inhibition; ➡ effects mediated. **From:** D. D'Ardes *et al.*, COVID-19 and RAS: Unravelling and Unclear Relationship, *Int J Mol Sci* 2020; 21(8): 3003

known for more than 50 years, only in the last decade the wide range of its physiological functions were unraveled.

In addition to the so-called somatic form (sACE) ACE1 (which consists of two homologous with independent catalytic structures, C-domain and the N-domain, of different biological functions (Fig. 2), two more forms have been discovered. The testicular form of the enzyme (tACE) consists of a C-domain and ACE2 monocarboxypeptidase isozyme that possesses one catalytic center and is not sensitive to inhibitors of ACE1 (4).

Figure 2. Schematic representation of the structural motifs of somatic (sACE) and testicular form (tACE) of ACE. Shown is the zinc binding motif illustrated with HExxH, present in both forms. **From:** Harrison, C., and Acharya, K.R. ACE for all – a molecular perspective. *J Cell Commun Signal* 2014; 8:195-210

Drugs widely used in medical practice, such as captopril, enalaprilat, trandolaprilat, lisinopril etc., which act as competitive inhibitors of ACE1, show a different affinity for both catalytic centers. It was reported that trandolaprilat binds best with C-domain, followed by lisinopril, enalaprilat, captopril (5). In contrast, the N-domain activity is in the order trandolaprilat > captopril > enalaprilat > lisinopril. These results show that selectivity of inhibition against both domains strongly depends on the structure of the inhibitor.

Besides participating in the regulation of the water-electrolyte balance (6) and systemic vascular resistance and blood pressure, renin-angiotensin system participates in functional modulation of many cellular systems and organs as the cognition processes (7), regulation of duodenal mucosa bicarbonate secretion (8), cellular differentiation and apoptosis (9), the inflammation processes (10) and male reproductive seminal function (11). It is interesting to mention the organ distribution of ACE1 activity in one of the most studied experimental animals, the rat. High specific activities of converting enzyme were found in lung and in segments of the digestive tract, but the highest activities were in testis and

epididymis, associated with tubular fluids, but not in sperm cells (12).

Over the past decade, the interest in biologically active peptides as potential therapeutic agents constantly increased due to diversity in their biological activity and significantly lower toxicity of the peptides and their breakdown products. An advantage of biologically active peptides is that they very rarely interact with other drug substances. The use of a specific peptide drugs is a potential alternative therapy for the treatment of hypertension (13). Along with this, an increased interest in foods and dietary supplements enriched with peptides with anti-hypertensive action.

Modern research is aimed to introduce new biochemical tests with the application of selective substrates to distinguish the activity of different ACE1 isoenzyme forms and clarification of their physiological functions. The results of these studies would substantially assist the search for new selective inhibitors with more effective structures (for the different ACE1 isoforms), such as- peptides, peptidomimetics, anthocyanins, flavonols and triterpenes.

2. ANGIOTENSIN CONVERTING ENZYME 2 (ACE2)

The ACE2, a single pass type I membrane monocarboxypeptidase, discovered 2 decades ago, consists of an N-terminal peptidase domain and C-terminal collectrin like domain (2, 14). It is the peptidase domain that is responsible for the main functions of RAS. The ACE2 shares 40 % homology with the N-terminal catalytic domain of ACE1, and a hydrophobic region near the C-terminus likely to serve as a membrane anchor. The ACE2 protein is encoded by the ACE2 gene located on chromosome Xp22. These ACE2 proteins are more abundantly expressed on the apical surface of the well-differentiated and mostly ciliated airway epithelium of the lungs (alveolar Type-2 cells), and enterocytes of the small intestine. Furthermore, ACE2 protein is expressed in arterial and venous endothelial cells and arterial smooth muscle cells, in the heart, kidneys, adrenal glands, pancreas, skeletal muscle, and adipose tissues.

ACE1 inhibitors (captopril, enalaprilat, lisinopril), widely used as drugs to regulate the level of angiotensin II and vascular tone, bind to the catalytic center of ACE2, without inhibiting it. It would be important to show whether these enzyme inhibitors can indirectly alter the conformation at the site of virus binding (RBD) and thus affect ACE2's interaction with the virus. It is certainly useful to test these drugs for their ability to block RBD-ACE2 interaction (15).

Data on influencing the functions of ACE2 in the body by

different xenobiotics are still insufficient. Some examples could be listed in this direction as:

Reducing the expression of ACE2 (from databases analysis (16):

- (i) Connectivity Map (CMap) – Azathioprine
- (ii) JeaMoon Map (JMap) (traditional Chinese medicine): Andrographis, Urtica, Sambucus, Astragalus, valproic acid, butyrate, and epoxomicin

ACE2 activators:

- (i) xanthenone (XNT): Chronic XNT administration improves the endothelial function of hypertensive and diabetic rat vessels by attenuation of the oxidative stress (17).
- (ii) resorcinolnaphthalein, a yellow dye (18)
- (iii) diminazene aceturate (an antitrypanosomal drug) (19).

ACE2 inhibitors:

- (i) DX600 (has K_i of 2.8 nM) is selective inhibitor. It is not hydrolyzed by ACE2 and it does not inhibit ACE activity (20).

3. ACE1, ACE2 AND SARS-COV-2

The coronavirus SARS-CoV-2, a single stranded RNA virus, has been seen to infect humans through their envelope spike glycoprotein (S-protein), which is responsible for CoV cell entry and host-to-host transmission. During viral infection, this S-protein cleaves into two fragments, S1 and S2. The FURIN cleavage site in the SARS-CoV-2S protein may provide a priming mechanism. The ectodomain S1 binds to the peptidase domain of the ACE2 enzyme, while S2 is cleaved further by the host cell serine protease TMPRSS2, resulting in membrane fusion. Both these steps are essential for the viral entry into cells.

Cardiovascular disease patients under ACE1 and ARBS blockers therapy

SARS-CoV-2 attacks any cell type in the body expressing ACE2.

What should the common people and the professionals be concerned about this fact, in the context of COVID-19 pandemic? (21). Extensive worldwide meta-analysis studies show that high blood pressure patients pose a higher risk to get afflicted with COVID-19, and are likely to have worse symptoms and to mortality rates.

The logical questions that follow are: Is this increased risk due to changes in health status of such patients (weaker

immune system) or is it due to the fact that they take medicines such as ACE1 inhibitors or angiotensin receptor blockers (ARBs)?

What are the evidence for beneficial or harmful effects of modulation of RAS by ACE1 inhibitors for the development and outcome of COVID-19 pneumonia?

It could be assumed that increased numbers of ACE2 receptors throughout their cardiopulmonary circulations may be the cause of the increased risk to get corona virus when taking ACE1 inhibitors or ARBS inhibitors. Indications to such effects were observed in experimental animal models (22).

The crucial evidence points towards the protective role of ACE1 inhibitors (but not ARBs) in patients subject to the risk of pneumonia. Patient populations that may benefit most are those with a history of stroke (23). ACE1 inhibitors were also associated with a decrease in pneumonia-related mortality (24). It is important to mention that the risk of pneumonia is reduced by about a third if ACE1 inhibitors are used for hypertension, as compared to the use of other antihypertensive drugs in patients with a history of stroke incidents (25).

Extensive meta-analysis enrolling 9890 hypertensive subjects strongly supports the recommendation of several scientific societies to continue ARBs or ACE1 inhibitors for all patients (26). The same positive conclusion was reached in a cohort study including 8.3 million people in UK. There was no evidence for any increased or reduced risk of COVID-19 patients in intensive care unit (ICU) admission treated with ACE1 inhibitor and ARB prescriptions (27). The same confirmative conclusions were reached by many others clinical studies (28-42).

With respect to striking an optimal balance between AT1R antagonists *versus* ACE1 inhibitors in patients with COVID-19, some studies stressed that it could be effective to prefer AT1R antagonists over ACE1 inhibitors in COVID-19 patients since sartans (Valsartan) maintain the function of the ACE2 enzyme, preserving the substrate Ang II, which is subject to be metabolized into Ang (1-7) (43).

Presently, all guidelines recommend continuing ACEI/ARBs in patients diagnosed with COVID-19 infection. Recommendations are as follows (44):

- (i) In non-infected patients and patients at risk, there is currently no valid reason to discontinue RAS blockade.
- (ii) In healthy subjects at risk, evidence is not (yet!) sufficient to prophylactically recommend RAS blockade.
- (iii) If apprehensions about increased infectivity persist, patients on ACEIs or ARBs could be switched

temporarily to a direct renin inhibitor.

- (iv) In COVID-19-positive patients on RAS blockers, the pharmacotherapy should be continued.
- (v) In febrile patients with pulmonary symptoms on RAS blockers, close monitoring of blood pressure and renal function is advisable; RAS blockers should be discontinued only as clinically indicated.

4. RAS, COVID-19 AND MALE AND FEMALE REPRODUCTION (45, 46)

Sperm is unique in containing the ACE C-domain isoform and in this regard, is a favorable site for testing the selectivity of inhibition of novel peptide compounds. Studying the effects of enzyme activity on human spermatozoa motility and comparing the effect of the new peptides inhibitors in this system would provide important information on the possible adverse effects of some ACE1 inhibitors used in medicinal practice. On the other hand, the activity of tACE could be proposed as a biomarker for the evaluation of the reproductive activity of human spermatozoa. A similar test was already proposed in veterinary medical practice for checking the reproductive capacity of bull spermatozoa (47).

Intra-cytoplasmic sperm injection (ICSI) is currently the primary technique used to achieve pregnancy when male infertility is a factor. Systemic clinical trials suggest that embryos with a higher implantation potential come from semen samples with higher percentages of testicular tACE-positive cells and fewer tACE enzyme molecules per spermatozoa (48).

ACE2 is regulated by a gene that is located on the X chromosome, thereby suggesting that some differences may exist in the expression of ACE2 in men and women. Men are more sensitive than women to coronavirus attack (49). It is suggested that this outcome is because low levels of androgens in women suppress TMPRSS2 (a serine protease) expression.

Human spermatozoa possess the entire repertoire of receptors (AT1R, AT2R, MAS) and ligand processing enzymes (ACE1 and ACE2) needed to support the angiotensin signaling cascade. The latter not only provides SARS-CoV-2 with a foothold on the sperm surface but may also promote integration, given the additional presence of a range of proteases (TMPRSS2, TMPRSS11B, TMPRSS12, furin) capable of promoting viral fusion.

What are the possible mechanisms by which corona virus affect male and female reproductive function? ACE2 receptors have recently been observed on human Leydig-Davidoff cells (50), implying a possible direct effect of the virus on the male

reproductive system. ACE2 receptors have been also reported to be expressed in human ovaries (51), while angiotensin-(1-7) has been detected in measurable amounts in the follicular fluid.

CONCLUSION

Since December 2019, the epidemic of SARS-CoV-2 has stimulated scientists around the world to examine the details of virus-host interaction. This is envisaged to lead to a successful therapeutic approach that could lead to reducing the morbidity and damage from the epidemic. In the efforts to test known drugs for their efficacy in a specific antiviral action, safety of the administration of ACE1 inhibitors in subjects have been explored. Several directions are relevant:

- (i) Does ACE-I interact with ACE2 as substrates or inhibitors? So far, all studies in this regard have been negative.
- (ii) Does ACE-I alter the affinity of interaction of ACE2 binding site with S-protein of viral head? No information so far.
- (iii) Increasing the demand for new selective inhibitors for different isoforms of ACE1, seeking to reduce adverse reactions (bradykinin induced cough; decreased male reproductive efficiency) and increase their effectiveness (higher inhibitory potency; increased oral bioavailability).

Accelerated and successful answers to these questions are possible only after the combined efforts of scientists from different fields of biology, pharmacy and medicine. The ideas and facts set out in this review reflect the efforts of such a unified scientific team.

CONFLICT OF INTEREST

The authors have no conflict of interests to declare.

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