Japan Journal of Medicine
2020; 3(2): 447- 451. doi: 10.31488/jjm.158

Research Article

Molecular Similarity Relating to a Peptide Sequence Common to Angiotensin II (ANGII) and SARS-Cov2 S-Protein

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Received: July 09, 2020; Accepted: July 14, 2020; Published: August 07,2020

Abstract

The peptidase entity of angiotensin-converting enzyme 2 (ACE2) accommodates the receptor-binding domain of SARS-COV2 spike (S) protein initiating cell infection. Compounds binding to ACE2 or the S-protein have the potential to impair cell entry by the virus. The ACE2 substrate angiotensin II (ANGII) shares common amino acid sequences with the S-protein. This study focuses on one sequence, the N-terminal valine/arginine/aspartate peptide of ANGII. Computer modeling identifies relative molecular similarity within the tripeptide sequence and ligands for ACE2, ANGII and sigma receptor proteins with in vitro toxicity for SARS-CoV2. Molecular similarity is also evident within drug structures of several neurotransmitter classes and agents from natural sources. Application of these observations may prove useful for the further development of SARS-CoV2 antiviral drugs.

Keywords: angiotensin-converting enzyme 2, angiotensin II, antiviral drugs, molecular structure; spike protein, SARS-CoV2, sigma receptor

Introduction

The affinity of SARS-CoV2 (Cv19) envelope proteins for angiotensin-converting enzyme 2 (ACE2) facilitates infection of human cells [1]. ACE2 cell receptors are present within several organ systems and expression in lung tissues (elevated in chronic smokers) increases significantly in airways epithelial cells infected with SARS-CoV [2]. There is 80% genome sequence identity between SARS-CoV2 and SARS-CoV1, with the latter's receptor-binding motif (RBM) for ACE2 comprising of residues 424-494 [3,4]. The amino acid sequence of the Cv19 Spike S1 receptor-binding domain (RBD) is available [5]. ACE enzymes and angiotensin peptides are essential components of the complex renin-angiotensin system (RAS) [1]. Individual susceptibility to Cv19 infection may be partially explained by natural variation within the different components of RAS [6].

ACE carboxypeptidases are ectoenzymes that cleave amino acid residues from the C-terminus of proteins. Angiotensin 2 (ANGII) an octapeptide (1-8) formed from angiotensin I (1-10) by ACE is subsequently depleted of amino acids by aminopeptidases to ANGIII and smaller peptides with various effects on blood pressure, aldosterone and sodium retention [7]. The ACE2 glycoprotein, integral to cell membranes, is anchored at the hydrophobic C-terminus with an extracellular N-terminal region containing the catalytic motif of 5 amino acid residues [8,9]. Catalysis of ANG (1-7) from ANGII substrate by ACE2 is completely inhibited in vitro by high concentrations of the C-terminal dipeptide, Pro.Phe [10]. ACE2 is also susceptible to inhibition or activation by other di- and tripeptides and naturally occurring small molecular weight compounds [9,11,12]. The primary physiologic actions of ANGII are mediated via the AT1 receptor (AT1R). The agonist adopts an extended structure within the binding pocket; N-terminal Val3 interacts with the second extracellular loop, whereas the C-terminus interacts with residues of the 7th transmembrane domain [13]. Asp1, Arg2, Tyr4, Phe8, determine the binding affinity and specificity of ANGII for AT1R, whereas Tyr4, His6, Pro7 and Phe8 account for agonist potency [14]. Less is known about the requirements for the recognition of ANGII by ACE2.

Several clinically promising antiviral di- and tripeptide drugs have been developed against HIV and Hepatitis C. Knowledge of the full amino acid sequences of ACE2 and SARS-CoV RBD and the application of sophisticated computational techniques have yet to provide blocking agents for clinical use [15]. An alternative approach focuses on drug repurposing, the testing of existing compounds for antiviral properties. AT1R blockers such as losartan provide one strategy to reduce the initial infective stage of Cv19 and resveratrol, a health supplement and dietary constituent, is another compound of interest [16]. Gordon and co-workers have recently identified a significant number of drugs that target interaction between viral and human proteins [17]. The drugs, including 4-7 residue peptides and cyclic peptides, have been assessed for their in vitro effects on the infection, growth and cytotoxicity of SARS-CoV2.

Notwithstanding the evidence supporting a link between the ACE2 receptor and SARS-CoV infection, histopathological

studies do not all support this mechanism [18]. The deficit of data on the relationship between ACE2 expression and Cv19 mortality, and the requirement for therapeutic agents to inhibit Cv19 infection informs the need for further research. Search of the S-protein sequence of Cv19 reveals two tripeptide sequences, valine/arginine/aspartate (VRD) and arginine/valine/tyrosine (RVY) each occurring twice within the protein [5]. These four amino acid residues are also present as the sequence aspartate/arginine/valine/tyrosine (DRVY) within the ANGII structure [19]. This study focuses on the VRD amino acid sequence common to ANGII and the S- protein of SARS-CoV2. A computer-aided modeling approach is applied to the investigation of molecular similarity within the structures of VRD, ligands of the ANGII system, sigma (σ) antiviral inhibitors and structurally similar compounds of potential interest.

Methods

The Nemesis software program (Oxford Molecular version 2.1) is used to build molecular structures from contents of the program fragment file and minimise structures by conformational analysis. The VRD peptide and compound structures are minimum energy conformers in an uncharged form. The computational program fits selected paired molecular structures on a three-point basis. Fitting-points comprise of atoms of similar type and partial charge within compound and peptide structures, identified in the figures with respect to amino acid residue labels. Compound colour-coded atoms in the figures identify ligand fitting-points: carbon-green, nitrogen-blue, oxygen-red, sulphur-yellow. To improve on presentation, bond order within the molecular structures is not shown and some peptide templates are repositioned with respect to each other to provide a better image of the compounds. The Nemesis program computes goodness-of-fit values, in respect of inter-atomic distance at each fitting point and root mean square (RMS) value.

Results

Table 1 lists the investigated compounds with their primary properties and fitting data. The data, encapsulating the fit of each structure to the val/arg/asp (VRD) residues of ANGII (Fig.1;2), demonstrate good fitting values with interatomic distances and RMS values respectively ≤0.16Å and ≤0.0200Å. The fits of the ATR ligands BMIC, losartan and L-162313 demonstrate the importance of an imidazole/imidazopyridine moiety (Fig.1; templates 3-6). In contrast to BMIC and losartan, L-162313 is an ATR agonist with a fitting-point that can be shared by its sulphonylcarbamate group and the carbonyl of valine (6). Alternative cyclic ring systems replace the imidazole moiety in structures other than camostat (7). The fits of resveratrol (11,12) are replicated by steroid hormone structures (9,10,13,14). Progesterone and dexamethasone provide the best steroid fits to VRD peptide and one that is replicated by testosterone (0.02Å, 0.08Å, 0.07Å; RMS 0.0069Å – not shown).

Compound fits to the peptide template in Figs. 2 and 3 generally follow the above pattern. Additional fitting characteristics of the structures (Fig. 2) include the C11 fitting point of rimcazole and the carbonyl group (C4O4) of haloperidol. In general, peptide fitting-points of the above compounds are found on two distinct cyclic rings. The first ring system has one or two

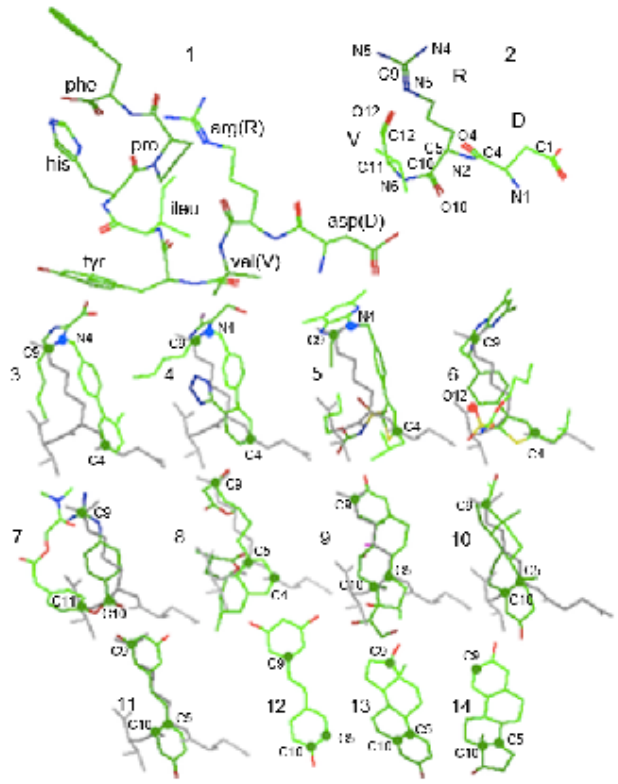

Figure 1. Angiotensin II (1) and N-terminal sequence of angiotensin II (2) with compound fits (3-14) to the N-terminal peptide template (grey): 3) BMIC, 4) losartan, 5) L-162313, 6) L-162313, 7) camostat, 8) lovastatin, 9) dexamethasone, 10) progesterone, 11) resveratrol, 12) resveratrol, 13) 17-β estradiol, 14) 17-β estradiol.

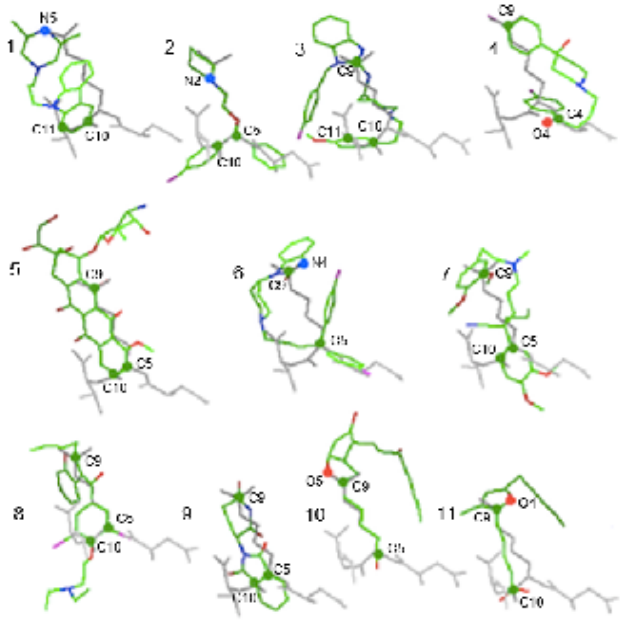

Figure 2. Compound fits (1-11) to the N-terminal peptide template (grey): 1) rimcazole, 2) cloperazine, 3) astemizole, 4) haloperidol, 5) doxorubicin, 6) pimozide, 7) verapamil, 8) amiodarone, 9) thalidomide, 10) prostaglandin I2, 11) MPFA.

fitting-points relating to the arginine guanidinium group and the second has fitting-points for peptide C4, C5 or C10. These cyclic rings are variously separated by a third cyclic ring, a short alkyl chain, or form part of a fused ring system in steroid-like structures. The various ring systems fitting to the arginine guanidinium group include aromatic and non aromatic rings (imidazole, piperazine, piperidine, chromone, furan) bicyclic rings (benzeneimidazo, indole, naphthalene, benzofuran) and larger ring systems (imidazopurine, tetracene). Templates 1-7 in Fig.2 include the structures of antiviral sigma (σ) receptor ligands identified by Gordon (daunorubicin is replaced here by doxorubicin) [17]. The peptide fitting-points of the σ ligands, including lovastatin and progesterone structures, are quite different in describing triangular pharmacophores of dimensions (Å) 1.25-2.5, 4.8-7.9, 6.3-8.1, in area 4.4 - 10.1 Å^2. Fig. 2 also gives the structures of the prostanoid PgI2 and natural lipid MPFA with fits based on a furan ring and carboxyl group (10,11).

Fig. 3 expands on the pharmacological diversity within the compounds investigated. These structures, including classical receptor agonists and antagonists and natural compounds with more general therapeutic properties, conform to fitting patterns identified in the previous figures. 5-hydroxytryptamine (5-HT) antagonists and the metabolite 5-hydroxyindoleacetic acid provide several different fits to the VRD peptide template. The ACE2 inhibitor structures AEAE and nicotianamine (11,12) differ in not fitting to the arginine guanidinium group and superimposing along the axis of the peptide chain. Of the compounds listed in Table 1, 70% fit solely to the arginine residue and 21% to the arginine-aspartate dipeptide.

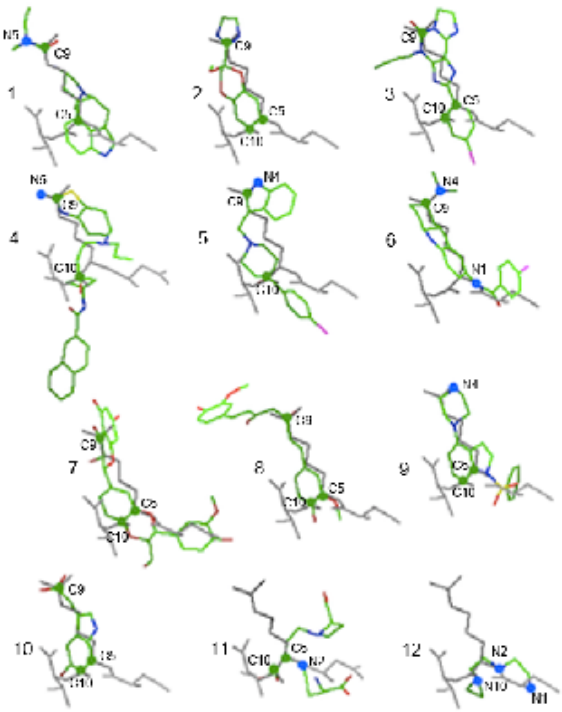

Figure 3. Compound fits (1-12) to the N-terminal peptide template (grey): 1) lisuride, 2) RX-821002, 3) KF-26777, 4) CJ-1639, 5) L-741626, 6) LY-344864, 7) silibinin, 8) curcumin, 9) BVT-5182, 10) 5-hydroxyindole acetic acid, 11) nicotianamine, 12) AEAE

Table 1. Fitting data of compounds and the val/arg/asp peptide

Compound	Target / descriptor	Fitting points	Interatomic distances (Å)	RMS (Å)
5-hydroxy-indole acetic acid	metabolite	C10C5C9	0.08,0.05,0.05	0.0012
17-βE	E	C10C5C9	0.03,0.16,0.14	0.0123
17-βE	E	C10C5C9	0.12,0.16,0.05	0.0175
AEAE	ACE2	N1N2O10	0.05,0.03,0.06	0.0137
amiodarone	ion channels, σ	C10C5C9	0.09,0.15,0.08	0.0143
astemizole	H	C11C10C9	0.09,0.05,0.10	0.0079
BMIC	AT	N4C9C4	0.04,0.05,0.01	0.0044
BV-T5182	5-HT	C10C5C9	0.09,0.10,0.14	0.0041
CJ-1639	D	N5C9C10	0.02,0.02,0.01	0.0021
camostat	serine protease	C11C10C9	0.06,0.06,0.05	0.0021
cloperastine	H, σ	C10C5N2	0.03,0.02,0.05	0.0019
curcumin	protective agent	C10C5C9	0.04,0.11,0.09	0.0065
dexamethasone	steroid	C10C5C9	0.01,0.04,0.04	0.0034
doxorubicin	anti-neoplastic	C9C5C10	0.08,0.15,0.08	0.0108
haloperidol	D, σ	O4C4C9	0.01,0.09,0.09	0.0040
KF-26777	A	C10C5C9	0.10,0.11,0.03	0.0114
L-162,313	AT	N4C9C4	0.01,0.14,0.13	0.0066
L-162,313	AT	C4O12C9	0.12,0.06,0.16	0.0200
L-741626	D, 5-HT	N4C9C10	0.03,0.09,0.06	0.0068
lisuride	α, D, 5-HT	N5C9C5	0.03,0.08,0.10	0.0040
losartan	AT	N4C9C4	0.08,0.01,0.09	0.0034
lovastatin	statin, σ	C4C5C9	0.05,0.01,0,04	0.0013
LY-344864	5-HT	N4C9N1	0.11,0.06,0.05	0.0060
MPFA	antioxidant	N4C9C10	0.09,0.06,0.15	0.0017
nicotianamine	ACE2	N2C10C5	0.08,0.07,0.06	0.0051
pimozide	D, 5-HT, H, σ	N4C9C5	0.09,0.03,0.10	0.0048
progesterone	E, σ	C10C5C9	0.02,0.08,0.07	0.0069
prostaglandin I2	prostanoid	N5C9C5	0.14, 0.08,0.20	0.0056
RX-821002	α	C9C5C10	0.03,0.09,0.11	0.0052
resveratrol	protective agent	C10C5C9	0.05,0.13,0.12	0.0069
reveratrol	protective agent	C10C5C9	0.04,0.12,0.13	0.0057
rimcazole	σ	C11C10N5	0.04,0.03,0.05	0.0017
silibinin	protective agent	C10C5C9	0.06,0.06,0.01	0.0030
thalidomide	anti-neoplastic	C9C5C10	0.03,0.12,0.11	0.0029
verapamil	ion channels, σ	C10C5C9	0.06,0.06,0.04	0.0018

Compounds: AEAE: N-(2-aminoethyl)-1-aziridineethanamine; BMIC: 5-butyl-methyl-imidazole carboxylate 30; MPFA: 3-methyl-5-pentyl-2-furan-pentanoic acid
Receptors: α: Adrenergic, σ: sigma; A: adenosine; AT: angiotensin, D: dopamine; E: estrogen; H: histamine; 5-HT: serotonin

Discussion

The results demonstrate relative molecular similarity within the structures of σ receptor ligands, compounds that target the angiotensin pathway, and a tripeptide sequence that contributes to the structures of ANGII and SARS-CoV2 S-protein. The VRD sequence is also shared by ANG1-9 and ANG1-7 but not by ANGIII. Molecular similarity within the arginine residue, statin and AT1R compounds is perhaps not surprising, as their therapeutic effects in RAS compromised patients are partly due to nitric oxide promoting properties [20]. AT1R blockers and resveratrol are recognized as potential SARS-CoV2 antivirals. Resveratrol displays inhibitory effects against several viruses, including MERS-CoV, and reduces RAS activity in rodent thoracic aorta and a model of nonalchoholic fatty liver disease [21-23]. AEAE and camostat inhibit the function of SARS-CoV S-protein and prevent Cv19 entry into cells [24,25].

Drugs with in vitro toxicity against SARS-CoV2 have been characterized as either translation inhibitors or σ receptor ligands [17]. The fits of compounds in the latter class (listed in Table 1) do not identify a common pharmacophore, as peptide fitting-points and distances between fitting-points are quite different. Although these compounds differ in structure, properties and fitting characteristics, they relate to each other through similarity to the peptide VRD. The σ receptor is described as an enigmatic evolutionary isolate, with poorly defined regulation by a remarkably diverse range of ligands [26]. There is a marked contrast between classical pharmacologic receptors and the protein targets of σ ligands that include enzyme structures. Pharmacophore mapping of over 8500 compounds in a GPCR-focused chemical library has recently created a model of the σ1 receptor, described by four key chemical features of the ligand-binding pocket alongside eight exclusion volume spheres [27]. Of the other drugs investigated here, verapamil, amiodarone, haloperidol, silibinin, genistein and thalidomide are active in inhibiting the infective or replicative phases of a variety of viruses [28-32]. Nicotianamine is an ACE2 inhibitor isolated from soybean whereas AEAE, a blocker of S-protein-mediated cell fusion, is a synthetic compound [9].

Several 5-HT antagonists and the metabolite 5-hydroxyindoleacetic acid relate to the VRD peptide structure. One surprising public health finding of the Cv19 pandemic is the low susceptibility of children to Cv19, raising the possibility of a natural inhibitor to infection within this age group. Interestingly, 5-HT synthesis is highest in childhood, especially between the ages of 2 and 5, declining to adult levels around 11 years of age [33,34]. Data obtained from rodent and cell culture systems demonstrate significant interaction between the serotonin and angiotensin systems. Serotoninergic dorsal raphe nucleus neurons are targets of ANGII; combined 5-HT/ANGII infusion reduces echocardiogram ejection fractions, causing a more aggressive remodeling of valves; blunting of platelet aggregation responses by ANGII relates to loss of the 5-HT transporter (SERT) [35-37].

Minor changes in ACE2 molecular structure are known to interfere with the binding of SARs-CoV. Whereas natural mutations within the RBD may not eliminate infection by SARS-CoV, a single point mutation at aspartate454 abolishes the association of the S1- protein with ACE2 [3,38]. Arginine and valine have been identified as variable residues within the RBDs of SARS-CoV and SARS-CoV2 that interface with ACE2 [3]. The S-protein domain contains in excess of 3% arginine residues with 16 valine-arginine sequences.

In conclusion, the data presented demonstrate that many drug structures with antiviral effects on SARS-CoV2 share molecular similarity with a peptide sequence within the S-protein, and the same characteristics are evident within compounds as yet untested for antiviral properties. This may be indicative of a common affinity for a viral receptor-protein but does not necessarily relate to the ACE2 binding site for the N-terminal residues of ANGII.

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To cite this article: Williams WR. Molecular Similarity Relating to a Peptide Sequence Common to Angiotensin II (ANGII) and SARS-Cov2 S-Protein. Japan Journal of Medicine. 2020; 3:2.