Structural insights into decreased affinity of SARS-CoV-2 to ACE2 by steroids

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Article

Abstract

The novel coronavirus disease (COVID-19) presently poses significant concerns around the world. Latest reports show that the degree of disease and mortality of COVID-19 infected patients may vary from gender to gender with a very high risk of death for seniors. Clearly, different levels of sex steroid hormones are found in both men and women. It was hypothesized that sex steroid hormones estradiol (E2), progesterone (P4), and testosterone (T) may change the interaction of coronavirus spike protein with angiotensin converting enzyme-2 (ACE2, which is the major SARS-CoV-2 cell entry receptor.) in the presence or absence of dexamethasone (DEX, the potential anti-inflammatory agents). Data showed that E2 was more strongly to interact with the main protease of the coronavirus, while T had the lowest affinity for coronavirus spike protein than E2 and P4. The binding energy of the spike protein to ACE2 was increased in the presence of five molecules of each steroid; the greatest increase was observed by DEX and E2. The binding free energy of the spike protein to ACE2 was the highest in the presence of both E2 and DEX molecules. Together, the interaction between spike protein and ACE2 can be disrupted by female sex steroid hormone E2 and to a greater extent by E2 and anti-inflammatory DEX, in part explaining the lower incidence of COVID-19 infection in women than men. The potential use of E2 and DEX to reduce coronavirus attachment to ACE2 in the early phase of the coronavirus invasion needs to be clinically investigated.

Introduction

Coronavirus pandemic was first recognized in the Hubei Province of China in December 2019 and has been reported as a very widespread disease with people-to-people transmission. Clinical data suggest that in terms of COVID-19, women are more resilient than men (*1-5*). In the previous research, the critical factors involved in increasing the mortality and severity of coronavirus in patients were investigated and it was found that there was a much more severe male problem (*2*). It has been reported that 12.8% of 86 men died and 75.6% recovered, while 7.3% of 82 females died and 86.6% recovered (*6*). In addition, the reports show a relatively low incidence risk for young people but a very high risk of death for seniors (*7*); Elderly patients were described as those diagnosed with COVID-19 aged 60 or older (*8*). Patients over 60 years of age showed greater clinical signs, higher severity and longer periods of illness (*9*).

Sex steroid hormones are the primary cause of female and male variations. Testosterone (T) identified as a predominant sex steroid hormone in males plays an essential role in sexual and reproductive development. In women, progesterone (P4) and estradiol (E2) are predominant sex steroid hormones and are produced by ovaries. With respect to the menstrual cycle, the concentration of E2 reaches the highest level just before ovulation (during the follicular or proliferative phase) and then decreases shortly afterwards (during the luteal or secretory phase). During the luteal phase, P4 is released at peak level, and then drops before the next menstrual period. Decreases in ovarian hormones associated with menopause have been well established (*10*). Women with the lowest concentrations of androgen and estradiol recorded worse quality of life scores (*11*).

Steroids, such as E2, P4, T, and dexamethasone (DEX), may be involved in inflammation and immune reactions (*12-15*). The expression of pro- and anti-inflammatory cytokines has been shown to change in the presence of steroid hormones (*12-15*). Bianchi found that a low level of T is involved in the production and regulation of pro-inflammatory cytokines (*13*). Hormone P4 has been shown to weaken sepsis syndrome by suppressing the production of inflammatory cytokines, such as IL-6 and TNFA (*14*).The effectiveness of corticosteroids, such as DEX, has been reported for the reduction of pro-inflammatory mediators (*16*). Corticosteroids may reduce mortality, need for mechanical ventilation, duration of mechanical ventilation, length of ICU stay and length of hospital stay for COVID-19 patients (*17*). Therefore, these findings clearly indicate the potential role of steroids in the regulation of COVID-19, in particular in females. Moreover, steroids due to the hydrophobic surface favor non-covalent interaction with a wide range of biomolecules, especially uncharged and aromatic amino acids (*18-20*). As a result, steroids can strongly bind to protein through hydrophobic interaction (*18, 19*).

Interaction between coronavirus spike protein and angiotensin converting enzyme-2 (ACE2) is widely recognized as a key step in coronavirus infection (*21, 22*). ACE2, an enzyme on the outer surface of the cells, plays a pivotal role in the entry of coronavirus into the cells (*5, 21, 23, 24*). Research on the affinity of ACE2 with the coronavirus protease or with the binding domain of spike protein can therefore shed some light on how to deal with the coronavirus pandemic. Protease is an essential enzyme found in viruses (*25*). The coronavirus 3-chymotrypsin-like protease (3CLpro), also known as Mpro, is the main protease, which is required for proteolytic maturation of the coronavirus (*25*). Protease enzyme catalyzes proteolytic reactions by cleaving covalent chemical bonds in proteins. Coronavirus protease has been reported as a target for many endogenous and exogenous inhibitors (*25, 26*). In fact, inhibition of protease is one the most important ways to treat coronavirus diseases (*25, 26*).

In this study, we hypothesized that, as endogenous inhibitors, predominant female sex steroid hormones (P4 and E2) may outperform T as the predominant male sex steroid hormone in reducing the affinity of coronavirus spike protein to ACE2. In addition, we attempted to understand the behavior of DEX in the presence of sex steroid hormones against coronavirus proteins interaction with ACE2. To this aim, computational approaches were used to determine a detailed molecular-level mechanism for the interaction of coronavirus spike protein with ACE2 in the presence of sex steroid hormones.

Results

Interaction of sex steroid hormones and dexamethasone with coronavirus protease and spike protein

The three-dimensional geometry of the ligand (steroid molecule)−receptor (coronavirus proteins) complex was obtained from the best docking coordinates (**Fig. 1**). The chemical structures of DEX and sex steroids T, P4 and E2 are shown in **Fig. S1**.

The binding energy value for the interaction of E2, P4, T and DEX with coronavirus protease and spike protein is shown in **Table 1.** Data showed that these steroid molecules had a strong binding affinity to

coronavirus protease and spike protein. The binding energy values ranged from -5.9 (4th and 5th T to spike protein) to -8.5 kcal/mol (5th E2 to the main protease) and strongly verified the interaction region between steroid molecules and coronavirus protease and spike protein (**Table 1**).

Compared to the primary male sex hormone T, the primary female sex steroid hormones E2 and P4 were more likely to interact with coronavirus spike protein (mean ± SD, -6.34 ± 0.54, -6.88 ± 0.43, and -6.88 ± 0.59 kcal/mol found for T, P4 and E2, respectively). Regarding the interaction between sex steroid hormones and the spike protein, the binding energy decreased from -7.1 to -7.8 kcal/mol (~10%) with an increase in E2 (**Table 1**). The binding energy increased from -7.2 to -5.9 kcal/mol (~ 18%) with an increase in T. For an increase in P4, the binding energy went up from -7.4 to -6.3 kcal/mol (~ 17.5%).

The female hormone E2 had the greatest tendency to interact with the protease enzyme (mean ± SD, -6.74 ± 0.43, -7.12 ± 1.42, and -7.40 ± 0.73 kcal/mol found for T, P4 and E2, respectively). In the case of the interaction between sex steroid hormones and the main protease, the binding energy decreased from -6.5 to -7.5 kcal/mol (~ 15.4%), with an increase in T (**Table 1**). The binding energy decreased from -7.2 to -7.7 kcal/mol (~ 6.9%) with increasing number of P4. For an increase in E2, the binding energy fell from -7.0 to -8.5 kcal/mol (~ 21.4%, **Table 1**).

Compared to sex steroid hormones, the value of binding energy was the minimum for DEX, (mean ± SD, -7.64 ± 0.23 and -7.11 ± 0.46 kcal/mol found for the interaction with the main protease and spike protein, respectively). In the case of the interaction between DEX and the main protease, the binding energy decreased from -7.5 to -7.7 kcal/mol (~ 2.7%), with an increase in DEX (**Table 1**). Regarding the interaction between DEX and spike protein, the binding energy went up from -7.8 to -6.7 kcal/mol (~ 14.1%), with an increase in DEX.

In order to analyze the distribution of amino acids at binding sites, docking data on residues involved in protein-steroid molecule interfaces was conducted. As seen in **Table 2**, the total number of residues involved in the interaction between steroid molecules and proteins was 117, including ~ 94.9 % (111/117) uncharged and ~ 5.1 % (6/117) charged residues. Moreover, the contribution of aromatic residues (Trp, Phe, and Tyr) was ~ 29.9 % (35/117).

Steroid molecules change the interaction of spike protein with ACE2

The binding free energy and the average hydrogen bond (H-bond) found between the spike protein and ACE2 are shown in **Table 3**. The calculated interaction energy of the spike protein with ACE2 was -2511.4kJ/mol, which clearly indicated a remarkable affinity (**Table 3A**). Steroid molecules appeared to be effective ligands in this system, due to an increase in the energy of binding. Compared to the basal interaction (spike protein-ACE2 interaction), the interaction of only five molecules of each steroid with spike protein increased the energy by 201.2 kJ/mol (8%), 192.4 kJ/mol (7.7%), 532.1 kJ/mol (21.2%), and 582.4 (23.2%) for T, P4, E2, and DEX, respectively (**Table 3B, C**). The H-bond analysis verified the devastating role of steroid molecules in interaction between spike protein and ACE2 (**Table 3**). The average H-bond over the last 10-ns MD simulation showed a sharp decrease with the docking of five

molecules of T (53.6%), P4 (27.5%), E2 (69.5%), or DEX (69.4%) to spike protein before the interaction of spike protein and ACE2.

In order to conduct a computational analysis of the spike protein/ACE2 binding site, a final snapshot of each complex was obtained from a 50 ns molecular dynamic (MD) simulation. As a result, the orientation of the spike protein closing to ACE2 was strongly dependent on the type of steroid molecules (**Fig. 2**). The important atoms and residues involved in the interaction are shown in **Figures S2- S5**. On the basis of the Ligplot analysis (**Figures S2-S5**), the number of effective residues in the spike protein-ACE2 complex binding site and the spike protein-5P4-ACE2 complex binding site was the highest compared to the other systems. In addition, the type of amino acids involved in binding interactions differed in all systems. As an example, Asp12 interacted as a negative charged amino acid of ACE2 (chain A) with Lys85 as a positive charged amino acid of spike protein (chain B) in the absence of hormonal influence. Upon docking of five E2 molecules, these amino acids were not selected as effective amino acid at the binding site. In the presence of five T molecules, Asp12 was bound to Arg76 as a positive charged amino acid of spike protein. This finding indicated that the binding site characteristics of spike protein were altered in the presence of steroid molecules.

In order to analyze the changes in the structure of the spike protein-ACE2 complex upon steroid hormone binding, the solvent accessible surface area (SASA) of the two proteins in the last 10-ns MD simulation was calculated and shown in **Fig. 3A.** The lowest SASA value was achieved for the spike protein-ACE2 complex compared to other complexes; more surface area of the complex was involved in the spike protein/ACE2 complex interaction. The low SASA value indicated a reduction in the number of water molecules covering the protein surface (**Fig. 3B**).

Compared to the basal interaction (spike protein-ACE2 interaction), the interaction of 5 molecules of DEX with spike protein increased the interaction energy between spike protein and ACE2 by 582.3 kJ/mol (23.2%, **Table 3C**). Also, the interaction of 5 DEX molecules with ACE2 increased the interaction energy between spike protein and ACE2 by 239.7 kJ/mol (9.5%) compared to the basal interaction (spike protein-ACE2 interaction, **Table 3C).** Binding of 5 DEX to spike protein before interaction with ACE2 could widen a considerable gap between spike protein and ACE2, as SASA values increased (**Fig. 3C**). However, the SASA was not dramatically changed in the case of binding DEX to the ACE2 and to the complex of spike protein-ACE2 complexes (i.e., spike protein /(5DEX-ACE2) or (spike protein-ACE2)/5DEX).

Radius of gyration (RG) of both spike protein and ACE2 indicated that docking T and P4 could make proteins more folded; however, E2 had a different effect (**Fig. 3D**). The spike protein/ACE2 complex tended to be more unfolding than the complexes in the presence of T and P4. The spike protein-5DEX-ACE2 complex changed the 3-D structure of the spike protein-ACE2 complex to be more unfolded (**Fig. 2, Fig. 3E**). By contrast, binding DEX to ACE2 or the complex made proteins folded. The result of SASA and RG verified the effect of steroid molecules on the spike protein/ACE2 complex structure.

In addition, the radial distribution function (RDF) analysis (the probability of spike protein in the distance *r* from ACE2) showed the strongest binding for spike protein/ACE2 complex (**Fig. 3F),** while both spike

protein-5E2-ACE2 ACE2 (**Fig. 3F)** and spike protein-5DEX-ACE2 complexes (**Fig. 3G)** had the weakest binding.

Combination of steroid hormones and dexamethasone, and their effect on the interaction of spike protein to ACE2

The three-dimensional geometry of the ligand (five steroid molecules, including E2, P4, T and DEX)-receptor (spike protein-ACE2 complex) is shown in **Fig. 2**. The changes in the interaction energy between the spike protein and ACE2 in the presence of 5 sex steroid hormones and 5 DEX were shown in **Table 3C.** As seen in **Table 3C,** the interaction energy between spike protein and ACE2 was the highest in the presence of DEX along with E2 compared to P4 and T (-994.3, -2009.1, and -2173.4 5 kJ/mol for E2, P4, and T). This indicated a sharp increase in the interaction energy of spike protein and ACE2 in the presence of DEX and E2 by 1517.1 kJ/mol (60.4%), compared to the basal interaction (spike protein-ACE2 interaction).

On the basis of the Ligplot analysis (**Figures S6 – S10)**, it can be clearly seen that the attachment of steroid molecules to spike protein, ACE2, or their complex altered the orientation and residues involved in the interaction. In absence of steroid molecules, nine important residues of ACE2 play a pivotal role in the interaction. Even though, the interaction of five steroid hormones and five DEX molecules reduced the number of residues to 3 and 6 for 5P4 + 5DEX and 5T + 5DEX, respectively. Also, the type of residue-residue interaction changed totally. As an example, Lys335 of ACE2 interacted with Gly164 and Gly170 of spike protein, but in the case of adding 5T + 5DEX, Lys335 interacted with Tyr121 of spike protein (**Fig. S10**). It should be noted that Ligplot analysis could not detect residues involved in the interaction when 5E2 and 5DEX were included; this was due to a large space that these molecules created between spike protein and ACE2.

Combination of steroid hormones and dexamethasone, and their effect on the structure of spike protein-ACE2 complex

In terms of the data of H-bond average, the number of h-bond plunged by means of docking of 5T + 5DEX (~ 64.8%), 5P4 + 5DEX (~ 62.4%) and 5E2 + 5DEX (~ 86.1%), as shown in **Table 3D**. The result of SASA clearly indicated that 5 sex steroid hormones and 5 DEX modified the 3-structure of the spike protein-ACE2 complex, while the 5E2+5DEX combination outperformed the other systems (**Fig. 4A**). The RG analysis clarified a sharp change in the structure of the spike protein-ACE2 complex upon interaction with a combination of 5E2 + 5DEX, causing the complex to be more unfolded (**Fig. 4B**). However, there was no major alteration in the structure of the complex after interaction with other systems (**Fig.4B**). In addition, the RDF analysis proved the adverse effect of the combination of sex steroid hormones and DEX on the spike protein/ACE2 interaction (**Fig. 4C**). With regard to 5E2 + 5DEX combination, there was less probability of spike protein atoms at a distance of r from ACE2, indicating a decreased affinity of spike protein to ACE2 (**Fig. 4C**).

Discussion

The findings of this study showed a very strong interaction between steroid molecules and coronavirus spike protein and protease, which might lead to a change in coronavirus function. The results showed that steroid molecules, including male and female sex steroid hormones and DEX, occupied different pockets of protein surface through non-covalent interaction. Hence, proteins showed a strong tendency to receive numerous steroid molecules. In addition, the protein sites occupied by steroid molecules shifted when confronted with these steroid molecules, suggesting that each molecule has its own protein binding site. However, the interaction of 5E2 and 5DEX increased the binding free energy more far than other complexes, i.e., 5T+5DEX or 5P4+5DEX complexes. Therefore, E2 and DEX were more effective in disrupting the binding of spike protein to ACE2 compared to P4 and T.

The present data showed a higher binding affinity of DEX and E2 to coronavirus main protease and spike protein, while T hormone as the main sex steroid hormone in men had the lowest affinity. DEX is known as a synthetic anti-inflammatory steroid and E2 is a predominant sex steroid hormone in the proliferative phase of the ovarian cycle in women. Moreover, we found that steroid molecules disrupt the interaction between the coronavirus spike protein and its receptor, ACE2. Importantly, ACE2 is a membrane protein located on the surface of the cells that facilitates the attachment of coronaviruses, including SARS, HCoV-NL63 and COVID-19, to host cell (*5, 21-24*). The interaction between ACE2 and coronavirus spike protein is an important aspect of infection (*5, 21-24*). In fact, this interaction is identified as a critical initial step to activate coronavirus penetration, allowing it to pass through the cell membrane (*5, 21-24*). The present results showed that both DEX and E2 molecules increased the binding energy between COVID-19 coronavirus spike protein and ACE2. This implied the potential role of DEX and E2 molecules in reducing the attachment of coronavirus spike protein to the ACE2 virus receptor. Recent studies have reported differences in the immune response of men and women to coronavirus infection (*1, 4-6, 12, 13*). Officials recorded a 2.8% fatality rate for male patients compared with 1.7% for female patients (*27*). However, the mechanism behind the gender difference remains unknown (*4, 28*), sex is a significant biological element to be considered for the prevention and treatment of COVID-19 (*4, 28*). The severity of influenza and other respiratory diseases has been reported to change in response to sex steroid hormones, such as estrogens (*29*). Using a simulation analysis, the present findings confirmed that the attachment of coronavirus to ACE2 may be adversely affected by steroid molecules, in particular DEX and E2 (which is the predominant sex steroid hormone in women during the follicular phase). Moreover, SASA analysis determined that steroid molecules can reduce the hydrophobic interaction between coronavirus spike protein and ACE2. Thus, steroid molecules, in particular DEX and E2, reduced the ACE2 surface of the contact site (increased SASA value) with the coronavirus spike protein, leading to a decrease in the cell penetration of the virus. As a result, DEX and E2 molecules may decrease the interactions between coronavirus and ACE2 by far more than other sex steroid hormones.

In the second part of this study, computational findings proved the cumulative effect of DEX and E2 on the interaction between coronavirus spike protein and ACE2. The presence of DEX and E2 strongly increased the binding free energy of spike protein and ACE2 (from -2511.4 to -994.3 kJ/mol). This clearly

indicated the potential benefit of DEX and E2 in reducing the attachment of coronavirus to ACE2 receptor. The results of SASA, and RG showed that the 5E2+5DEX combination highly changed the 3-strucure of the spike protein-ACE2 complex and caused this complex to be more unfolded. In addition, the RDF analysis confirmed the adverse impact of 5E2 + 5DEX combination on the spike protein/ACE2 interaction such that there was less probability of spike protein atoms at a distance of r from ACE2. This finding indicates a decreased affinity between spike protein and ACE2. As data showed, the combination of 5E2 + 5DEX had the highest H-bond percentage (~ 86.1%) with spike protein-ACE2 complex. This, in turn, causes this complex to be more unfolded, indicating that 5E2 + 5DEX readily forms hydrogen bonds with the protein backbone and disrupts native contacts (30). These findings may explain, at least in part, that why females show a lower rate of infection with COVID-19 than males.

DEX as a low-cost steroid medication is typically used to reduce inflammation in COVID-19 patients needing ventilation (31). Recent evidence suggests that a subset of patients with severe COVID-19 may have cytokine storm syndrome (31) and early administration of short-term corticosteroids improves the clinical outcomes of patients with severe COVID-19 pneumonia and evidence of immune hyperreactivity (32). High dose and short-term corticosteroid therapy early in respiratory failure have been reported to provide good prognosis for patients with COVID-19 (33). Nonetheless, some clinical evidence does not support corticosteroid therapy for 2019-nCoV lung injury and high-dose corticosteroids cannot necessarily be prescribed for the treatment of COVID-19 (31).

In conclusion, this finding suggested that E2 adversely affects the structure of coronavirus spike protein and its interaction with ACE2. Moreover, the present findings implied that the use of DEX can be more effective in the presence of E2. Therefore, the attachment of coronavirus to ACE2 and response of body to DEX can be changed by higher levels of E2 in women during the follicular phase. Moreover, the determination of the ovarian phase in women infected with COVID-19 should be studied in order to understand whether this helps to predict the extent of infection and the potential response of female patients to DEX. This may also help to assess the need for individual and pathophysiological treatment of patients. In addition, more research is required on the protection and possible benefits of E2 for men diagnosed with COVID-19. Importantly, cellular activity is changed in response to different physiological or pathophysiological conditions (34). Therefore, further studies are required to examine the interactive effects of certain physiological or pathophysiological factors on the degree of coronavirus attachment to ACE2 and the severity of infection in response to sex steroid hormones.

Methodology

Design of the computation study

Phase I: Docking simulation of steroid molecules and coronavirus proteins

Molecular docking was used to detect the precise molecular-level mechanism for the interaction of steroid molecules with the main important proteins of coronavirus, spike protein and protease. Using docking simulation programs, researchers are able to estimate the binding free energy between ligands

and receptors and predict interaction sites (*35, 36*). Scientifically, the biological activity of the protein is heavily dependent on its three-dimensional structure under physiological or pathophysiological conditions (*37, 38*). In fact, the biological activity of proteins may be impaired by blocking their active or binding sites by means of toxins, endogenous and exogenous molecules, hormones, medicines, etc (*34, 38, 39*). Therefore, we intended to conduct research on the potential affinity of male and female sex steroid hormones and DEX to coronavirus protease and spike protein. **Fig. S1** shows the chemical structures of DEX, T, P4 and E2.

The structure of coronavirus spike protein (PDB ID: 6LZG) and protease (PDB ID: 6LU7), T, P4, and E2 was selected to predict binding energy and binding site using AutoDock VINA (*40*). In this study, T, P4 and E2 without any rotatable bond were considered as ligands to be docked with coronavirus spike protein and protease. The grid sizes of 60 × 60 × 60 and 64 × 80 × 60 points along the x and y and z axes with grid spacing of 1Å were selected for spike protein and main protease as the receptor grid box, respectively. Docking procedure was performed five times in a row, so that each time the previous docked ligand/receptor complexes were used as the receptor for the next docking run.

Phase II: MD simulation of the spike protein-ACE2 complex in the presence of sex steroid hormones and dexamethasone

Docking simulation was conducted on the spike protein/ACE2 complex in the presence of zero to five docked steroid molecules using the Haddock web server (*41*). The complex of spike protein with five steroid molecules (obtained from phase I, spike protein-5 steroid molecules) and ACE2 (PDB ID: 6LZG) were the two proteins applied to HADDOCK web server. The active residues at the binding site of two proteins have been listed as follows: spike protein (Ala43, Lys85, Asn155, Gln161, Gly164, Gln166, Thr168, Gly170, Tyr173, Gln174) and ACE2 (Asp12, Gln307, Ser1, Lys13, Glu19, Tyr23, Lys335, Asp20, Tyr65). The MD simulation and the MM-PBSA approach were then used to calculate the binding free energy of coronavirus spike protein to its receptor, ACE2 (*42, 43*). In addition to what was mentioned above, 5DEX was first docked to ACE2 (via AutoDock VINA, with a large grid size to cover whole molecule) and then the binding free energy between spike protein and ACE2 was calculated using MD simulation and MM-PBSA approaches.

Phase III: MD simulation of spike protein/ACE2 complex in the presence of sex steroid hormones and dexamethasone

To study the effect of combination of steroid hormones and DEX on the interaction between spike protein and ACE2, docking and MD simulations were applied separately. In the first step, the complex structures of ACE2/(spike-5 steroid hormones) obtained from the phase II (Haddock web server) and DEX without any rotatable bond were selected as the receptor and ligand in turn for docking simulation. Docking procedure was performed five times in a row; each time the previous docked ligand/receptor complex was used as the receptor for the next docking run. Large grid box was made to cover the whole receptors (ACE2/(spike-5 steroid hormone)) for each docking simulation.

MD simulation details

All MD simulations in this project were performed in four steps for free molecules in the water box. Some Na$^+$ and Cl$^-$ ions were added to neutralize the system. In the first step, the entire system was minimized using the steepest descent algorithm and the process included 50000 cycles without any position restrictions. In the second and third steps, the equilibration process was completed by molecular dynamics at the 100 ps NVT set followed by the 100 ps NPT set by the restriction of proteins and sex steroid hormones at the 1000 kJ/mol·nm^{-2} harmonic force constant in the NPT phase. In the final step or production step, 50 ns MD simulations were carried out without any position restrain. The TIP3P water model was used to design the solvation box of molecules with a distance of 1.5 nm between the solute and the box walls. The simulations were performed at a temperature value of 300 K with a time step of 2 fs, taking into account the periodic boundary condition (PBC) in equilibration and production processes, and employing GROMACS 2020 with CHARMM 27 force field parameters (*44, 45*). Topology of steroid molecules was obtained from the SwissParam Web-site based on the CHARMM force field parameters (*46*).

Declarations

Author contributions: Conceptualization: R.K, K.S. Investigation: A.M., R.K. Resources: A.M., R.K., K.S. Writing: A.M., R.K. Supervision: A.M., R.K.

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Data and Materials Availability: All data needed to evaluate the conclusions in the paper are present in the paper or the Supplementary Materials.

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Tables

Table 1. The binding energy values.

Steroid	Simulation	Main protease	Spike protein
T (E_{min})	1st	-6.5	-7.2
	2nd	-6.5	-6.5
	3rd	-6.5	-6.2
	4th	-6.7	-5.9
	5th	-7.5	-5.9
P4 (E_{min})	1st	-7.2	-7.4
	2nd	-7.1	-7.2
	3rd	-6.8	-6.8
	4th	-6.8	-6.7
	5th	-7.7	-6.3
E2 (E_{min})	1st	-7.0	-7.1
	2nd	-6.9	-6.7
	3rd	-6.8	-6.5
	4th	-7.8	-6.3
	5th	-8.5	-7.8
DEX (E_{min})	1st	-7.5	-7.8
	2nd	-7.4	-7.3
	3rd	-8.0	-6.7
	4th	-7.6	-7.1
	5th	-7.7	-6.7

The minimum binding energy (E_{min}, kcal/mol) was obtained from the nine best docking simulations using the Vina docking algorithm of different steroids in the coronavirus protease and spike protein. T: testosterone; P4: progesterone; E2: estradiol; DEX: dexamethasone.

Table 2. The residues involved in protein-steroid interaction.

The main protease	T	1st	Trp218, Phe223, Glu270
		2nd	Met 165, Glu166, Gln189
		3rd	Val104, Gln110, Asn151, Ser158, Phe294
		4th	Pro293, Phe294
		5th	Phe294, Val297
	P4	1st	Leu272, Leu287, Glu288
		2nd	Met165, Glu166, Gln189
		3rd	Trp218, Asn221, Phe223, Glu270
		4th	Ile100, Val104, Asn151
		5th	Val104, Asp153
	E2	1st	Val104, Asn151, Phe294
		2nd	Phe219, Asn221, Phe223, Glu270, Asn274
		3rd	Gln107, Gln110, Ile249
		4th	Pro293, Phe294
		5th	Ile249
		1st	Thr25, Met49, Asn142, Gly143, Met165
		2nd	Ile106, Gln110, Asn151, Phe294
	DEX	3rd	Gln110, Ile249
		4th	Phe294, Val297
		5th	Tyr154, Phe294
Spike protein	T	1st	Pro426, Phe464, Ser514
		2nd	Phe338, Phe342, Leu368

	3rd	Arg403, Tyr453
	4th	Asn343, Thr345, Leu441
	5th	Phe392, Thr430, Leu517
P4	1st	Pro463, Phe464, Ser514
	2nd	Phe338, Phe342, Asp364, Val367, Leu368
	3rd	Phe392, Thr430, Leu517
	4th	Arg403, Gly496, Tyr505
	5th	Lys378, Val407, Arg408
E2	1st	Gly339, Phe342, Val367, Leu368, Ser373, Phe374
	2nd	Asn343
	3rd	Phe464, Glu516
	4th	Gly502, Tyr505
	5th	Asp501
	1st	Phe338, Asn343, Val367, Leu368, Phe374
	2nd	Phe464
DEX	3rd	Asn343, Trp436, Leu441
	4th	Arg346, Leu441, Asn448
	5th	Tyr495, Tyr505

In order to analyze the distribution of amino acids at binding sites, docking data on residues involved in protein-hormone interfaces was conducted. The total number of residues involved in the interaction between steroid molecules and proteins was 117, including ~ 94.9 % (111/117) uncharged and ~ 5.1 % (6/117) charged residues. Moreover, the contribution of aromatic residues (Trp, Phe, and Tyr) was ~ 29.9 % (35/117). T: testosterone; P4: progesterone; E2: estradiol; *DEX: dexamethasone.*

Table 3. Binding free energy and the average number of hydrogen bonds.

Complexes	Binding free energy (kJ/mol)	Hydrogen bonds (Average)
1. Basal interaction (Spike protein-ACE2)	-2511.4	11.52
1. Coronavirus spike protein interaction with ACE2 in the presence of sex steroids		
(Spike protein-5T)/ACE2	-2310.2	5.34
(Spike protein-5P4)/ACE2	-2319.0	8.35
(Spike protein-5E2)/ACE2	**-1979.3**	3.52
1. Coronavirus spike protein interaction with ACE2 in the presence of dexamethasone (DEX)		
(Spike protein -5DEX)/ACE2	**-1929**	*3.87*
Spike protein/(5DEX-ACE2)	-2271	*8.53*
(Spike protein-ACE2)/5DEX	-2264	*10.08*
1. Coronavirus spike protein interaction with ACE2 in the presence of sex steroids and DEX		
(ACE2-(Spike protein-5T))/5DEX	-2173.4	*4.05*
(ACE2-(Spike protein-5P4))/5DEX	-2009.1	*4.33*
(ACE2-(Spike protein-5E2))/5DEX	**-994.3**	*1.95*

The binding free energy of spike protein to ACE2 was obtained from the MM/PBSA methods and the average number of hydrogen bonds (H-bonds) was evaluated between coronavirus spike protein and ACE2 during the last 10-ns MD simulation (from 40 ns to 50 ns). T: testosterone; P4: progesterone; E2: estradiol; S: spike protein; A: ACE2 (a*ngiotensin-converting enzyme 2); DEX: dexamethasone; 5: number of steroid molecules (i.e., estradiol, or...).*