

Effects of Sex and Androgenic Drugs on the Expression of Angiotensin-Converting Enzyme 2 Receptor, Cathepsin I and Transmembrane Serine Protease in Mouse Lungs

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Abstract

Introduction: Although males and females have the same prevalence of COVID-19, a variation in the severity of symptoms between males and females has been observed. We hypothesize that this variation can partly be explained by the effect of androgens on the infectious activity of the SARS-Cov2 virus.

Aims: This study investigated the effect of sex and two androgenic drugs testosterone and oxandrolone on the mRNA expression of several SARS-Cov2 entry genes: angiotensin-converting enzyme 2 (ACE2), transmembrane protease serine 2 (TMPRSS2), and cathepsin I (CatL) in mouse lungs.

Methods: Twenty-eight BALB/c mice were divided into four groups; the first three groups (all male mice) were treated with the vehicle, testosterone, and oxandrolone, respectively, while the fourth group consisted of untreated female mice. The androgenic drugs were administered for 21 days in doses equivalent to the human one. Accordingly, the expressions of ACE2, TMPRSS2, and CatL genes were measured using real-time PCR assay. In addition, the histopathological alterations in the lungs and the levels of total serum testosterone were analyzed.

Results: We found that the expression of ACE2 was significantly upregulated in the lungs of the testosterone-treated group by 2.5 times. The expression of TMPRSS2 was also significantly upregulated in the lungs of oxandrolone-treated mice by 6.6 times. Moreover, these molecular alterations were associated with a high elevation of the serum testosterone and the induction of inflammation and oxidative stress. In addition, we found that the mRNA levels of ACE2, TMPRSS2, and CatL were significantly higher in the lungs of the female compared to male mice.

Conclusion: We found several significant differences between the mRNA expression of ACE2, TMPRSS2, and CatL genes in the lungs of male and female mice. We showed how the administration of testosterone and oxandrolone to male mice upregulated the lungs' mRNA expression of ACE2 and TMPRSS2, respectively. These results can expand our molecular understanding of the roles of sex and androgenic drugs on the expression of SARS-Cov2 entry genes.

Keywords: ACE2, lung, oxandrolone, testosterone, TMPRSS2, SARS-Cov2, COVID-19

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by the severe respiratory syndrome coronavirus 2 (SARS-Cov2) [1–2]. The SARS-Cov2 virus mainly infects the respiratory system but can spread and affect many vital organs causing numerous symptoms, such as dyspnea, dry cough, high fever, headache, vomiting, and diarrhea [3]. The symptoms can vary in degree, ranging from no symptoms (asymptomatic) to mild, moderate, or severe symptoms that lead to hospitalization [1, 3].

The SARS-Cov2 is a single-strand RNA virus enveloped in a spherical shape with crown-like antigens on its surface. It mainly consists of four structural proteins: a spike, membrane, envelope, and nucleocapsid

proteins, with the spike proteins facilitating the viral entry inside the host cells (Figure 1) [4]. The human angiotensin-converting enzyme 2 (ACE2) receptor is the targeted receptor for the SARS-Cov2 virus on the host cell surface [4], and the lung is the most affected organ in the body, partly due to the high expression of the human ACE2 receptors on the lung's epithelial cells [5]. SARS-Cov2 attaches to the human ACE2 receptor through its spike, and the activation of the spike protein is triggered by a two-step cleavage process mediated by human transmembrane protease serine 2 (TMPRSS2) and cathepsin 1 (CatL) [4, 6]. Accordingly, human ACE2, TMPRSS2, and CatL are the major proteins involved in the SARS-Cov2 entry into the host cells.

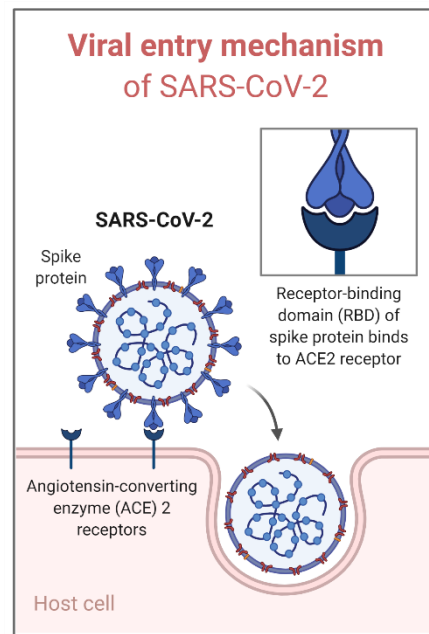


Figure 1. The role of spike proteins in SARS-Cov-2 entry mechanism. Adapted from BioRender.com (2022)

Epidemiological data suggest a significant sex difference in the severity of COVID-19. According to Klein et al., the incidence of the disease between females and males is the same, but the outcomes of the disease in males are much worse in comparison to females [7]. Moreover, it has been seen that the mortality rate is higher in males compared to females

among SARS-Cov2 infected patients [8], and males and females may develop different outcomes from COVID-19 due to the differences in their hormonal levels and the function of their immune system [9].

In this study, we decided to focus on the effect of anabolic-androgenic steroids as they are a large group of synthetic derivatives of

testosterone, used to increase anabolic effects, such as masculinization [10]. One of the most widely used synthetic anabolic-androgenic steroids is oxandrolone [11–13], an orally administered drug approved by the Food and Drug Administration to restore weight after disease-induced weight loss, surgery, chronic infections, and severe trauma [13]. There is a lack of in-vivo studies regarding the influence of sex and androgens on the expression of SARS-Cov2 entry genes in the lung. We hypothesized that there are differences in the expression of SARS-Cov2 entry genes ACE2, TMPRSS2, and CatL in the lung among males and females and that some anabolic drugs, such as oxandrolone, can alter the expression of these genes. Therefore, we investigated the influence of sex and androgenic drug administration on the mRNA expression of the mouse ACE2, TMPRSS2, and CatL genes in the lung.

MATERIALS AND METHODS

Chemicals

Testosterone, 250 mg/ml injection under the brand name Sustanon was obtained from Sabbagh Drug store (Amman, Jordan). Oxandrolone, a 10 mg tablet under the brand name Anavar was purchased from Pharmacom Labs (Chişinău, Moldova). Polyethylene glycol 400 was obtained from Guangdong Guanghua Sci-Tech Co., Ltd. (Guangdong, China). TRIzol solution (CAT number: 15596026) and a cDNA synthesis kit (CAT number: K1612) were obtained from Thermo Fisher Scientific (Massachusetts, USA). Luna® Universal qPCR Master Mix was brought from New England Biolabs (Massachusetts, USA). Lastly, the PCR primers were obtained from Integrated DNA Technologies (Coralville, Iowa, USA).

Experimental animals

Twenty-eight BALB/c mice (*Mus musculus*) of the same age (7–8 weeks old) and weights of 20–35 g were obtained from the animal house of Al-Zaytoonah University (Amman, Jordan). All mice were handled according to the ethics of animal laboratories as in Canadian guidelines [14]. They were kept in a temperature-controlled setting (23±1°C) with a 12-hour

light/12-hour dark cycle and free access to specific animal feed pellets.

Experimental protocol

Before drug administration, the mice were housed for a 7-day period for acclimation. Then, they were separated into four groups, according to their sex and the type of drug they would receive. Each group contained seven mice, as follows:

- i. The first group was the control, in which male mice received 50% polyethylene glycol 400, the vehicle used to dissolve the androgenic drugs;
- ii. The second group of male mice received 3.5 mg/kg oxandrolone, which was dissolved in 50% polyethylene glycol 400;
- iii. The third group of male mice received a therapeutic dose of testosterone 45 mg/kg dissolved in 50% polyethylene glycol 400;
- iv. The fourth group of female mice received no medications.

All mice were given the drugs as bi-weekly intraperitoneal route (IP) injections for three weeks, with an injection volume of 0.2mL. The usual human maintenance dose of the testosterone injection is one injection of 250 mg/1mL every 3 weeks [15]. However, the intervals between the doses and the number of doses administrated in comparison with the human dose were adjusted in consideration of the shorter lifecycle of mice. The doses of the drugs used in this research are similar to the human equivalent dose and adjusted based on the surface area of the animal's body [16]. After 21 days of drug administration, the mice were euthanized and the lung samples isolated for molecular and histological analyses. Blood samples were also collected once on the last day of the experiment, from each male mouse, for further biochemical analyses.

Physical observation

We conducted daily follow-ups to check on the animals and record whether there had been deaths or any major changes. The weight of the mice was measured twice, first at the beginning of the study and then following the full course of treatment (day 21 of IP injections) just before the mice were euthanized. The weight gain of the mice was calculated as the difference in the weights between the second (21 days) and first

time points.

Histological analysis

The lung tissues were dissected and washed in 0.9 percent normal saline before being immersed in a 10% formalin solution for days. The samples were then passed through a graded series of alcohol washes followed by xylene washes to achieve the appropriate dehydration before being preserved in pure paraffin wax. For the staining of the lung sections, hematoxylin and eosin were used, and the prepared sections were photographed by a Leica® microscope.

Serum testosterone measurement

The level of free serum testosterone for each male mouse was measured by enzyme-linked immunosorbent assays (ELISA) (IDS, Tyne & Wear, UK) (CAT number: IS-5300) with a minimum detection limit of 0.1 ng/ml. The testosterone level for each mouse was analyzed on the last day of administration of androgenic drugs and after animal scarification.

RNA extraction and cDNA synthesis

Total RNA extraction started with the isolation of about 200 mg of fresh lung tissue, followed by the addition of 1 mL of triazole solution, 200 L of 20% chloroform, 500 L of isopropyl alcohol, 500 L of 75 percent ethanol, and 10 L of nuclease-free water to the isolated lung samples, as instructed by the manufacturer. The extracted mRNA was then

converted to cDNA using the cDNA Synthesis Kit®. One ng total RNA was added to a reaction mixture containing 100 pmol oligo deoxythymine, 2.5 mM dNTP, 0.1 M DTT, 1X reverse transcriptase buffer, and 100 units of Moloney murine leukemia virus reverse transcriptase, and incubated for 60 minutes at 37 °C.

Gene expression analysis

The mRNA expressions of mouse ACE2, TMPRSS2, and CatL genes were examined in this research. The primer sequence, the annealing temperature, and the amplicon size for each amplified gene are summarized in Table 1. The quantitative real-time polymerase chain reaction was used to measure the expression of these targeted genes (RT-PCR), as prescribed [17–18]. Briefly, 80 ng of the synthesized cDNA was mixed to a reaction mixture of Luna® Universal Master Mix and 10 pmols of forward and reverse primers. The PCR conditions used were as follows: denaturation at 95 °C for 3 minutes followed by 40 cycles of denaturation at 95 °C for 10 seconds and annealing at 53–58 °C for 30 seconds (Table 1). The housekeeping gene in this study was glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and the expressions of the genes were determined using the $\Delta\Delta CT$ method [19]. The experiments were performed in triplicate and repeated twice.

Table 1. The primer sequence, the amplicon size, and the annealing temperature of ACE2, CatL, TMPRSS2, and GAPDH genes

Gene name	Forward	Reverse	Size	Annealing temp
ACE2	ATTCACCCAACACTTGAGCC	TGTCCATCGAGTCATAAGGG T	213	55
CatL	AGGAAAATGGAGGTCTGGAC T	GCAACAGAAATAGGCCCCAC	205	58
TMPRSS2	CGTTCCCGTATACTCCAGGT	CGTTCCCGTATACTCCAGGT	221	58
GAPDH	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA	450	53

In-silico analysis of the promoter sequence

The androgen receptor binding elements (AREs) and estrogen receptor binding elements were predicted in the promoter sequences of

mouse ACE2 and TMPRSS2 and human ACE2 using the PROMO online tool (v. 8.3 of TRANSFAC, Spain) [20]. The DNA sequence of the promoter region of mouse ACE2, mouse TMPRSS2, and human ACE2 was obtained

from Eukaryotic Promoter Database (<https://epd.epfl.ch/index.php>) [21]. The promoter DNA sequence used in this in-silico prediction, was 1,000 nucleotides before the transcriptional start site in the 5'-flanking region of human ACE2, mouse ACE2 and mouse TMPRSS2 genes. The alignment of the promoter sequences of human ACE2 with mouse ACE2 was performed with MultAlin (<http://multalin.toulouse.inra.fr/multalin/>) [22].

Statistical analysis

A one-way ANOVA test and then a post hoc Tukey HSD analysis were used for the comparison of the numerical data between the groups. The results were considered significant

when the p -value was less than 0.05.

Results

Physical observation

Figure 2 shows the change in mice weight of all tested groups between day 1 and day 21 using a two-way ANOVA test. It is evident from Figure 2 that there is a significant increase in the weight of the mice in both the testosterone and the oxandrolone treated groups (p -value<0.05). The average weight gain of the mice treated with testosterone and oxandrolone increased by 3.5 and 8.1 mg, respectively. Finally, we did not notice a significant difference (p -value>0.05) in the weight gain between female and control male mice.

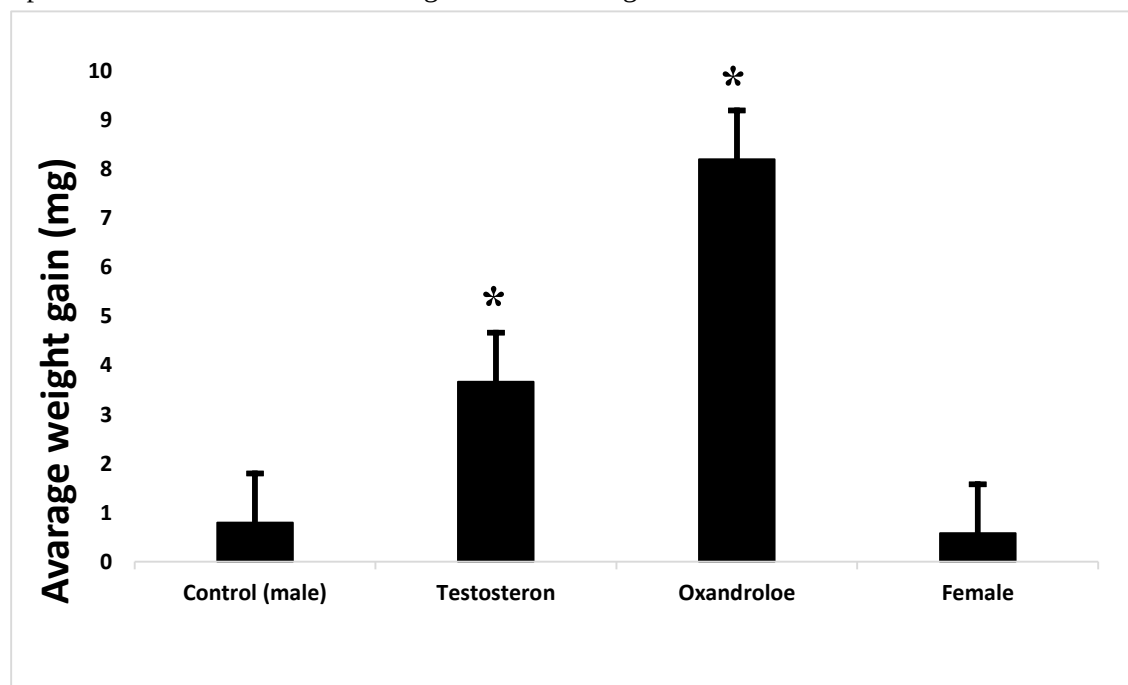


Figure 2. The average weight gain of the experimental mice. Data are presented as mean $\pm$ standard deviation of 7 mice in each group. * indicates a statistical alteration (p <0.05, one-way ANOVA test). The weight gain of the mice was calculated as the difference in the weights between days 1 and 21

Histological analysis

The histological sections of the mouse lung after the administration of testosterone and oxandrolone are shown in Figures 3 A–D. No histological alterations in the lungs of the control male mice were observed (Figure 3 A), but in the groups which received exogenous

testosterone some pathohistological alterations were observed in the appearance of inflammatory cells (Figures 3 B and C). In addition, the lungs of the mice that received oxandrolone showed more accumulation of inflammatory cells (Figure 3 D) in comparison to the testosterone group.

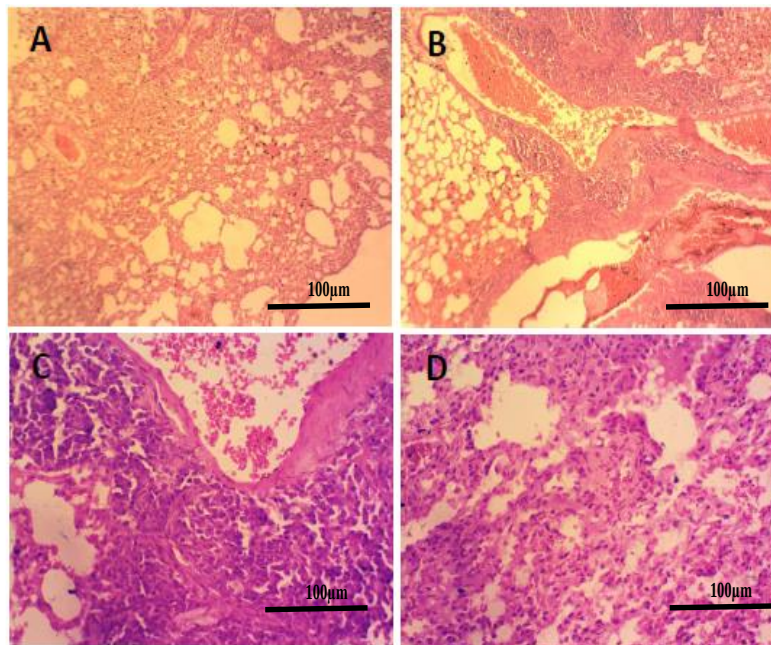


Figure 3. Histopathologic lungs analysis of animals after drug administration. A: control lung section shows the normal structure of the bronchiole and adjacent alveoli. B and C: testosterone-administrated mice representative lung section showing pathohistological alteration represented as inflammations. D: oxandrolone-administrated mice lung tissue showing more accumulation of inflammatory cells in the lung epithelium tissue. Tissue sections were stained with H&E (scale bar = 100 µm) and photographed at 40X magnification

Analysis of testosterone level

It is clear from Figure 4 that there was a significant increase in the serum levels of testosterone hormone of the mice after administration of the androgenic drugs, exogenous testosterone, and oxandrolone. The serum level of the testosterone hormone increased

significantly (p -value<0.05) in both the testosterone- and oxandrolone-treated mice by 177.85 and 15.16 times, respectively, in comparison with the control mice. Furthermore, the level of testosterone was significantly (p -value<0.05) higher among the testosterone group compared to the oxandrolone-treated mice.

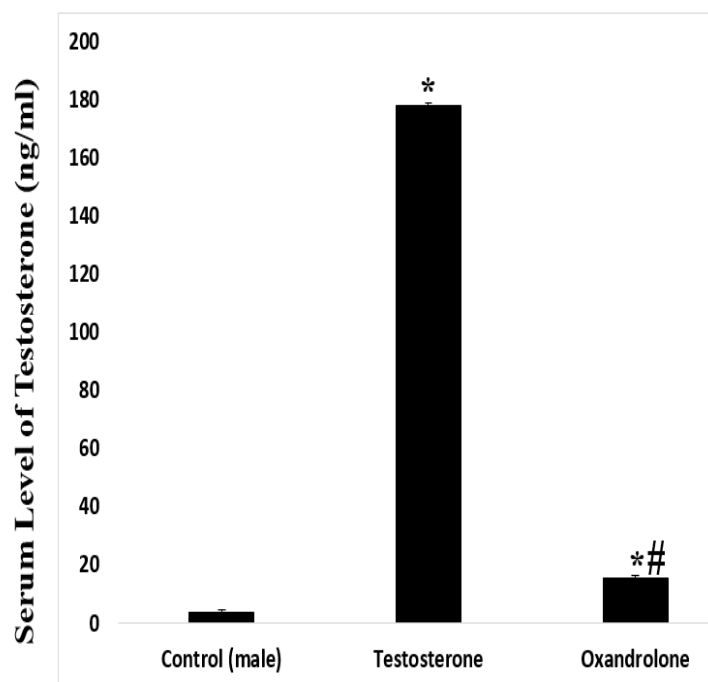


Figure 4. The level of testosterone hormone between the three male mice groups. The testosterone level for each mouse was analyzed on the last day of administration of androgenic drugs and after animal scarification. Data are presented as mean $\pm$ standard deviation. * indicates a statistical alteration (p <0.05, One-way ANOVA test), while # indicates a significant difference between oxandrolone and testosterone-treated mice

mRNA levels of SARS-Cov-2 entry genes

In this study, we found that the gene expression of SARS-Cov2 genes was affected significantly by the sex of the mice as well as the administration of the androgenic drugs. The expression of SARS-Cov2 entry genes was significantly higher in the lungs of females compared to males. The expressions of ACE2, TMPRSS2, and CatL were significantly higher in female lungs by 3.2, 3, and 2.5 times, respectively (Figures 5A–C). Moreover, the testosterone

treatment significantly upregulated (p -value<0.05) the expression of the ACE2 gene by more than two times, but it did not affect the expression of TMPRSS2 and CatL genes (Figures 5A–C). Oxandrolone treatment significantly upregulated (p -value<0.05) the expression of the TMPRSS2 gene by more than six times (Figure 5B), but it did not affect the expression of ACE2 and CatL genes (Figures 5A & C).

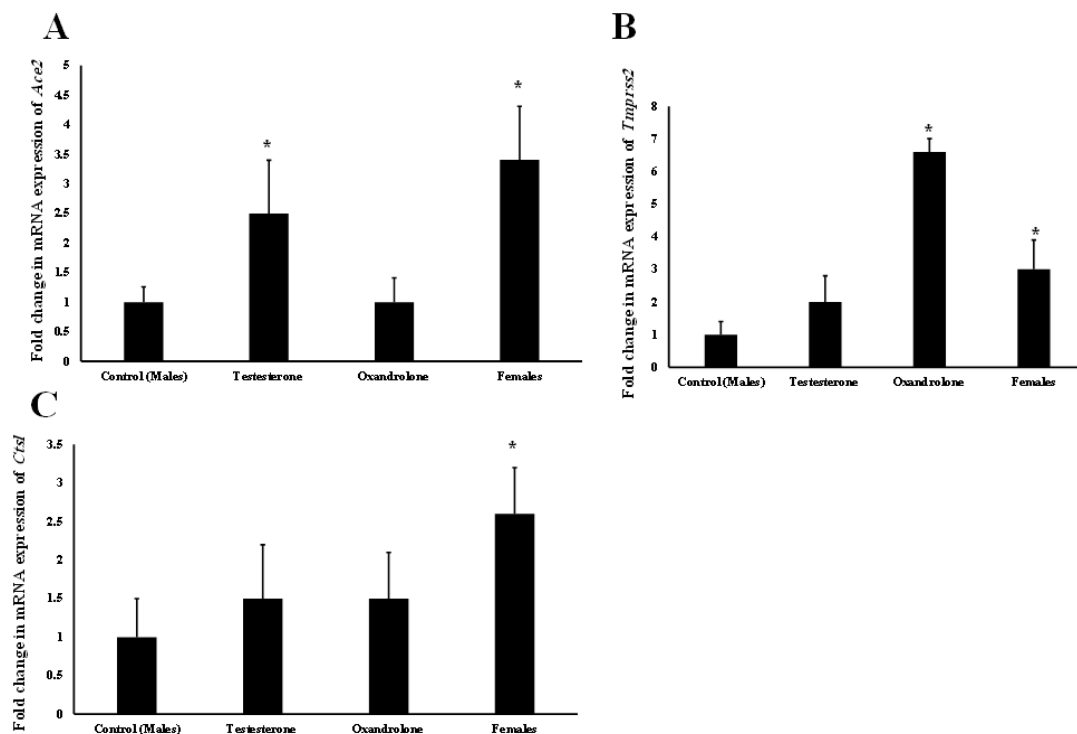


Figure 5. Relative fold change in mRNA expression of ACE2 (A), TMPRESS2 (B), and CatL (C) in the mouse lungs. Data are presented as mean $\pm$ standard deviation of 7 mice in each group. * indicates a statistically significant alteration (p <0.05, one-way ANOVA test).

Experiments were performed in triplicate and repeated twice

In-silico prediction of AREs in the promoter sequences

Table 2 presents the prediction of AREs in the promoter sequence of mouse ACE2 and TMPRSS2 genes. Different sequences of AREs

(CTGTTCT, GGTGAACA, and AAGGAACA) were identified in the promoter of ACE2, while only one ARE (AGAACTG) was identified in position -981 to -987 of the DNA promoter sequence of TMPRSS2 gene.

Table 2. Prediction of AREs in the promoter sequence of mouse ACE2 and TMPRSS2 genes

Gene	Position of ARE in the promoter #	Sequence of ARE
Mouse ACE2	-880 to -886	CTGTTCT
	-695 to -702	AAGGAACA
	-311 to -318	GGTGAACA
Mouse TMPRSS2	-981 to -987	AGAACTG

The position of AREs in the promoter sequence is relative to the transcriptional start site in ACE2 and TMPRSS2 genes

DISCUSSION

COVID-19 is continually spreading and mutating around the world, and many researchers are investigating the differences in the incidence and severity of the symptoms; moreover, sex is considered a contributing factor to these differences. Klein et al. [7] found that the incidence of COVID-19 in females and males was the same, but other studies reported men having a higher incidence of the disease than females [8–9]. These previous studies also pointed out that sex hormones might affect the infectious activity of SARS-Cov2 and ultimately the severity of the COVID-19 infection. In this study, we have investigated the expression of ACE2, TMPRSS2, and CatL genes, which play major roles in the entry of the SARS-Cov2 into the epithelial cells of the lungs. We also investigated the effect of the administration of exogenous testosterone and oxandrolone on the expression of these genes in the lungs of male mice. The results showed that female mice had higher expression of SARS-Cov2 entry genes in comparison to male mice. Additionally, administration of androgenic drugs to the male mice upregulated the mRNA expression of ACE2 and TMPRSS2 genes, which were associated with induction of inflammation in the lungs and elevation of serum testosterone levels. These results help explain, at least partly, the molecular influence of androgens on the SARS-Cov2 entry genes.

Wang et al. suggested that increased expression of TMPRSS2 and ACE2 genes can enhance the entry of the SARS-Cov2 virus into the epithelial cells in the lung and hence increase the severity of COVID-19 symptoms [23]. In this study, we found that mRNA expression of the ACE2 gene was significantly upregulated in the lungs of male mice

administrated with exogenous testosterone hormone. In addition, the expression of the TMPRSS2 gene was significantly upregulated in the lungs of male mice after the administration of oxandrolone. Accordingly, it can be suggested that androgenic drugs upregulate the expression of ACE2 and TMPRSS2 genes and hence increase the ability of SARS-Cov2 to enter the pulmonary cells and the risk of COVID-19 infection. For example, one report showed that the severity of COVID-19 symptoms was increased in a patient who was on oxandrolone treatment [24]. Furthermore, through using in-silico and in-vitro assays, it has been found that androgens can upregulate, while estrogens downregulate, the expression of human ACE2 and TMPRSS2 [1, 25–26]. Our results are consistent with these previous studies, which indicate that both androgenic drugs upregulated the expression of mouse ACE2 and TMPRSS2. However, we found that female mice had higher expression of ACE2, TMPRSS2, and CatL compared to males. This finding is inconsistent with the results of Stelzig et al. [26], a study which reported that estrogens downregulate the mRNA expression of human ACE2 using in-vitro assays. This might be due to an inter-species difference in the regulation of mRNA expression of human ACE2 and mouse ACE2 by estrogens or due to other factors that need further investigation. We analyzed 1,000 base pairs of the promoter sequence of human ACE2 and mouse ACE2 genes, using MultAlin software

(<http://multalin.toulouse.inra.fr/multalin/>), and found that there are differences in the promoter sequences (Supplementary Figure 1) and hence the binding elements of the transcriptional factors of both genes. Interestingly, the binding

sites of estrogen receptor alpha and beta were identified in the promoter sequence of human ACE2 but were not found in the promoter sequence of mouse ACE2 using in-silico prediction by PROMO software (Supplementary Table 1). Therefore, it might be speculated that there is a difference in the regulation of mRNA expression by estrogens between human ACE2 and mouse ACE2 genes.

Cattrini et al. suggested that using anti-androgenic drugs may have a protective role against COVID-19 [1]. It can be speculated from the results of this study that anti-androgenic drugs might ameliorate the androgenic induced changes of the expression of ACE2 and TMPRSS2. The molecular mechanism of how androgenic drugs upregulate the mRNA expression of ACE2 and TMPRSS2 is still not fully understood. Therefore, we analyzed, in this study, the promoter regions of mouse ACE2 and TMPRSS2 genes using PROMO software and found multiple binding sites for androgen receptor in these regions. This further suggests that the activation of androgen receptors in the pulmonary cells by androgenic drugs might play a role in the upregulation of ACE2 and TMPRSS2 genes. Further experiments are still needed to determine how androgens can upregulate the expression of ACE2 and TMPRSS2 genes.

There is a difference in the chemical structure between oxandrolone and testosterone which affect the pharmacokinetics and efficacy of both drugs. Oxandrolone is more lipophilic, less metabolized in the liver, and has more anabolic than androgenic activity, in comparison with testosterone [27]. This chemical difference in the structure of oxandrolone and testosterone might also affect the interaction between both drugs with the transcriptional factors, or other regulatory factors of mouse ACE2 and TMPRSS2 genes; it could also explain, at least in part, the gene-specific regulation of the mouse ACE2 and TMPRSS2 genes by androgens.

The present study showed that both testosterone and oxandrolone significantly (p -value<0.05) increased the average weight gain of the treated subjects. However, oxandrolone had a stronger influence on weight gain than the

administration of exogenous testosterone. This can be explained in terms of the ratio of the anabolic to androgenic effect of oxandrolone being more than three times higher than the anabolic/androgenic ratio of testosterone [21, 28]. Moreover, histological examinations showed that the administration of both testosterone and oxandrolone caused pathological alterations in the mouse lungs. Both androgenic drugs in this study induced inflammatory responses in the epithelial cells that might be a response against the oxidative stress of oxandrolone and exogenous testosterone on the lung cells [29]. These findings are supported by previous studies which showed that androgens can cause oxidative stress on different organs, including the lungs [30–31], conflicting with another study which reported that testosterone and oxandrolone have anti-inflammatory effects on the lungs [32]. Accordingly, it might be suggested that the administration of oxandrolone and testosterone to SARS-Cov2 infected patients is a potential risk factor for lung toxicity and may worsen the pulmonary symptoms of COVID-19 [22].

Finally, it is important to note that one of the limitations of our study is that we used BALB/c mice that were not infected by SARS-Cov2. However, we focused on the molecular influence of androgenic drugs on mouse ACE2, TMPRSS2, and CatL genes, which have close nucleic and amino acid sequences to the human ones, and we found molecular relationships that could link androgens to COVID-19 severity. This in-vivo study opens the door for further experiments on humanized ACE2, and TMPRSS2 mouse models [33] to confirm our findings and provide a better understanding and extrapolation of the effects of androgens on human SARS-Cov2 entry genes.

CONCLUSION

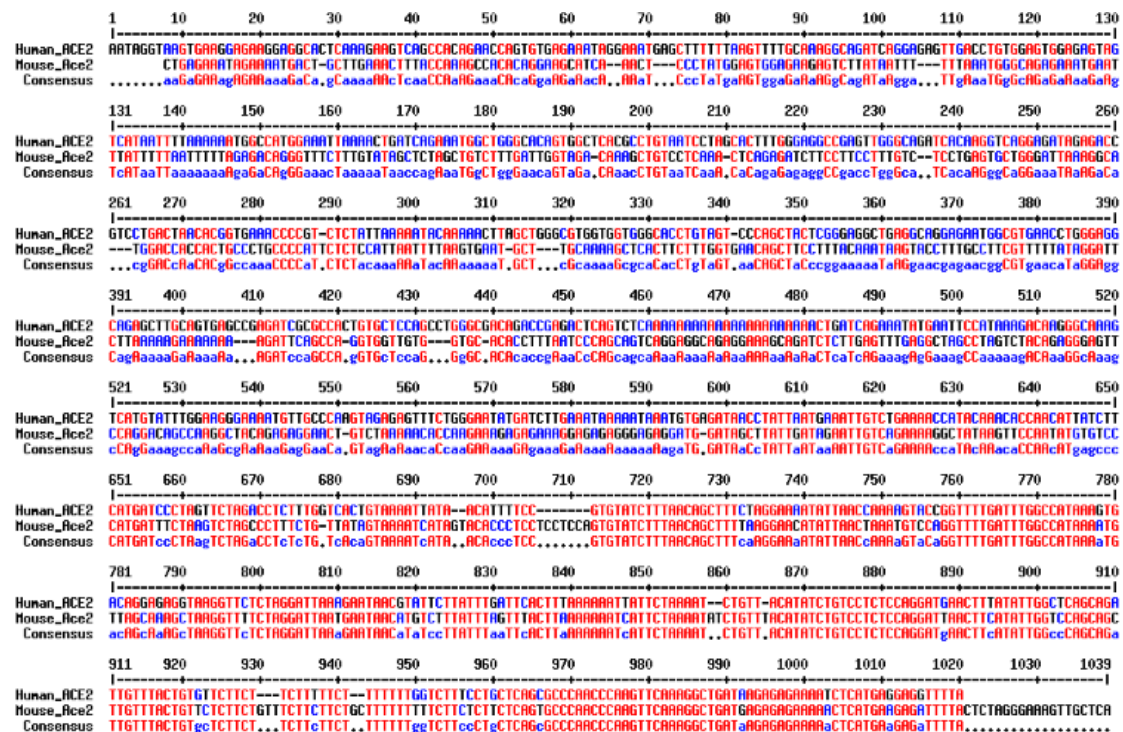
We investigated the influence of sex and some androgenic drugs on the expression of ACE2, TMPRSS2, and CatL genes and correlated the expression with the pathological alterations in mice lungs. The results showed significant differences in the lung mRNA

expression of ACE2, TMPRSS2, and CatL genes between male and female mice. In addition, testosterone and oxandrolone upregulated the mRNA expression of ACE2 and TMPRSS2 genes and induced oxidative stress in the mouse lungs. These findings increase our understanding of the molecular

mechanism of sex and androgenic drugs on the ACE2 receptor and proteases involved in the entry of SARS-Cov2 into host cells.

Funding

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Supplementary Figure 1. The alignment of 1,000 nucleotides of the promoter sequences of human ACE2 and mouse ACE2 genes. The alignment was performed using MultAlin software (<http://multalin.toulouse.inra.fr/multalin/>). The red color indicates that there is a similarity while the black and blue colors of the nucleotides indicate a difference in the promoter sequences

Supplementary Table 1. Prediction of estrogen receptor α and β binding elements in the promoter sequence of human ACE2 and mouse ACE2 genes

Gene	Position of estrogen receptor- α binding site in the promoter #	Sequence of estrogen receptor binding site	Position of estrogen receptor- β binding site in the promoter #	Sequence of estrogen receptor binding site
Human ACE2	-108 to -115	TTGACCTG	-109 to -117	TGACCTGTG
	-241 to -248	AAGGTCAG	-239 to -247	ACAAGGTCA
	-673 to -680	TTGGTCAC	-671 to -679	CTTTGGTCA
Mouse ACE2	---	Not found	----	Not found

The position of AREs in the promoter sequence is relative to the transcriptional start site in human ACE2 and mouse ACE2 genes

REFERENCES

- Cattrini C, Bersanelli M, Latocca MM, Conte B, Vallome G, Boccardo F: Sex Hormones and Hormone Therapy during COVID-19 Pandemic: Implications for Patients with Cancer. *Cancers* (Basel). 2020; 12(8). 10.3390/cancers12082325.
- Almomani EY, Qablan AM, Atrooz FY, Almomany AM, Hajjo RM, Almomani HY: The Influence of Coronavirus Diseases 2019 (COVID-19) Pandemic and the Quarantine Practices on University Students' Beliefs About the Online Learning Experience in Jordan. *Front Public Health*. 2020; 8:595874. 10.3389/fpubh.2020.595874.
- Sharma A, Tiwari S, Deb MK, Marty JL: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2): a global pandemic and treatment strategies. *Int J Antimicrob Agents*. 2020; 56(2):106054. 10.1016/j.ijantimicag.2020.106054.
- Mahmoud IS, Jarrar YB, Alshaer W, Ismail S: SARS-CoV-2 entry in host cells-multiple targets for treatment and prevention. *Biochimie*. 2020; 175:93-98. 10.1016/j.biochi.2020.05.012.
- Beyerstedt S, Casaro EB, Rangel EB: COVID-19: angiotensin-converting enzyme 2 (ACE2) expression and tissue susceptibility to SARS-CoV-2 infection. *Eur J Clin Microbiol Infect Dis*. 2021; 40(5):905-919. 10.1007/s10096-020-04138-6.
- Mahmoud IS, Jarrar YB: Targeting the intestinal TMPRSS2 protease to prevent SARS-CoV-2 entry into enterocytes-prospects and challenges. *Mol Biol Rep*. 2021; 48(5):4667-4675. 10.1007/s11033-021-06390-1.
- Klein SL, Dhakal S, Ursin RL, Deshpande S, Sandberg K, Mauvais-Jarvis F: Biological sex impacts COVID-19 outcomes. *PLoS pathogens*. 2020; 16(6):e1008570.
- Pradhan A, Olsson PE: Sex differences in severity and mortality from COVID-19: are males more vulnerable? *Biol Sex Differ*. 2020; 11(1):53. 10.1186/s13293-020-00330-7.
- Scully EP, Haverfield J, Ursin RL, Tannenbaum C, Klein SL: Considering how biological sex impacts immune responses and COVID-19 outcomes. *Nat Rev Immunol*. 2020; 20(7): 442-447. 10.1038/s41577-020-0348-8.
- Hartgens F, Kuipers H: Effects of androgenic-anabolic steroids in athletes. *Sports Med*. 2004; 34(8):513-554. 10.2165/00007256-200434080-00003.
- Abed AF, Jarrar YB, Al-Ameer HJ, Al-Awaida W, Lee SJ: The Protective Effect of Metformin against Oxandrolone-Induced Infertility in Male Rats. *Curr Pharm Des*. 2022; 28(4):324-330. 10.2174/1381612826666201029101524.

- 12 Hammad AM, Ibrahim YA, Khdaif SI, Hall FS, Alfaraj M, Jarrar Y et al: Metformin reduces oxandrolone- induced depression-like behavior in rats via modulating the expression of IL-1beta, IL-6, IL-10 and TNF-alpha. *Behav Brain Res.* 2021; 414:113475. 10.1016/j.bbr.2021.113475.
- 13 Kindermann W, Urhausen A: Left ventricular dimensions and function in strength athletes. *Re: Hartgens F, Cheriex EC, Kuipers H. Prospective echocardiographic assessment of androgenic-anabolic steroids effects on cardiac structure and function in strength athletes. Int J Sports Med* 2003; 24: 344 - 351. *Int J Sports Med.* 2004; 25(3):241-242; author reply 243-244. 10.1055/s-2004-817850.
- 14 Rowsell HC: The Canadian Council on Animal Care--its guidelines and policy directives: the veterinarian's responsibility. *Can J Vet Res.* 1991; 55(3):205.
- 15 Klaver M, de Mutsert R, Wiepjes CM, Twisk JWR, den Heijer M, Rotteveel J et al: Early Hormonal Treatment Affects Body Composition and Body Shape in Young Transgender Adolescents. *J Sex Med.* 2018; 15(2):251-260. 10.1016/j.jsxm.2017.12.009.
- 16 Nair AB, Jacob S: A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm.* 2016; 7(2):27-31. 10.4103/0976-0105.177703.
- 17 Jarrar YB, Jarrar Q, Abed A, Abu-Shalhoob M: Effects of nonsteroidal anti-inflammatory drugs on the expression of arachidonic acid-metabolizing Cyp450 genes in mouse hearts, kidneys and livers. *Prostaglandins Other Lipid Mediat.* 2019; 141:14-21. 10.1016/j.prostaglandins.2019.02.003.
- 18 M AA, Jarrar Y: Effects of vitamin C and D on the mRNA expression of angiotensin converting enzyme 2 receptor, cathepsin L, and transmembrane serine protease in the mouse lungs. *Libyan J Med.* 2022; 17(1):2054111. 10.1080/19932820.2022.2054111.
- 19 Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods.* 2001; 25(4):402-408. 10.1006/meth.2001.1262.
- 20 Farre D, Roset R, Huerta M, Adsua JE, Rosello L, Alba MM et al: Identification of patterns in biological sequences at the ALGGEN server: PROMO and MALGEN. *Nucleic Acids Res.* 2003; 31(13):3651-3653. 10.1093/nar/gkg605.
- 21 Perier RC, Praz V, Junier T, Bonnard C, Bucher P: The eukaryotic promoter database (EPD). *Nucleic Acids Res.* 2000; 28(1):302-303. 10.1093/nar/28.1.302.
- 22 Corpet F: Multiple sequence alignment with hierarchical clustering. *Nucleic Acids Res.* 1988; 16(22):10881-10890. 10.1093/nar/16.22.10881.
- 23 Wang Q, Li L, Qu T, Li J, Wu L, Li K et al: High Expression of ACE2 and TMPRSS2 at the Resection Margin Makes Lung Cancer Survivors Susceptible to SARS-CoV-2 With Unfavorable Prognosis. *Front Oncol.* 2021; 11:644575. 10.3389/fonc.2021.644575.
- 24 Cadegiani F, Lin EM, Goren A, Wambier CG: Potential risk for developing severe COVID-19 disease among anabolic steroid users. *BMJ Case Rep.* 2021; 14(2). 10.1136/bcr-2021-241572.
- 25 Strobe JD, Chau CH, Figg WD: Are sex discordant outcomes in COVID-19 related to sex hormones? *Semin Oncol.* 2020; 47(5):335-340. 10.1053/j.seminoncol.2020.06.002.
- 26 Stelzig KE, Canepa-Escaro F, Schiliro M, Berdnikovs S, Prakash YS, Chiarella SE: Estrogen regulates the expression of SARS-CoV-2 receptor ACE2 in differentiated airway epithelial cells. *Am J Physiol Lung Cell Mol Physiol.* 2020; 318(6):L1280-L1281. 10.1152/ajplung.00153.2020.
- 27 Handelsman DJ: Androgen Physiology, Pharmacology, Use and Misuse. In: *Endotext.* edn. Edited by Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatriya K, Dungan K, Hershman JM, Hofland J, Kalra S et al. South Dartmouth (MA); 2000.
- 28 Kicman AT: Pharmacology of anabolic steroids. *Br*

- J Pharmacol. 2008; 154(3):502-521.
10.1038/bjp.2008.165.
- 29 Yang L, Liu G, Lin Z, Wang Y, He H, Liu T et al: Pro-inflammatory response and oxidative stress induced by specific components in ambient particulate matter in human bronchial epithelial cells. *Environ Toxicol.* 2016; 31(8):923-936. 10.1002/tox.22102.
- 30 Alonso-Alvarez C, Bertrand S, Faivre B, Chastel O, Sorci G: Testosterone and oxidative stress: the oxidation handicap hypothesis. *Proc Biol Sci.* 2007; 274(1611):819-825. 10.1098/rspb.2006.3764.
- 31 Osgood RS, Kasahara DI, Tashiro H, Cho Y, Shore SA: Androgens augment pulmonary responses to ozone in mice. *Physiol Rep.* 2019; 7(18):e14214. 10.14814/phy2.14214.
- 32 Wang X, Huang L, Jiang S, Cheng K, Wang D, Luo Q et al: Testosterone attenuates pulmonary epithelial inflammation in male rats of COPD model through preventing NRF1-derived NF-kappaB signaling. *J Mol Cell Biol.* 2021; 13(2):128-140. 10.1093/jmcb/mjaa079.
- 33 Sefik E, Israelow B, Mirza H, Zhao J, Qu R, Kaffee E et al: A humanized mouse model of chronic COVID-19. *Nat Biotechnol.* 2021. 10.1038/s41587-021-01155-4.

تأثيرات الجنس وأدوية الهرمونات الذكورية على التعبير الجيني للمستقبلات و الإنزيمات المسؤولة عن دخول فايروس SARS-Cov2 إلى الرئة في الفئران المخبرية

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الملخص

المقدمة: في حين أن الذكور والإناث لديهم نفس معدل انتشار COVID-19 ، إلا أنه لوحظ تباين في شدة الأعراض بين الذكور والإناث. نفترض أن هذا الاختلاف يمكن تفسيره جزئياً بتأثير الهرمونات الذكورية على قدرة العدوى لدى فيروس-SARS Cov2 المسبب لعدوى كورونا.

الأهداف: هدفت هذه الدراسة إلى معرفة مدى تأثير الجنس والعقاقير المحتوية على الهرمونات الذكورية: التستوستيرون والأوكساندرون على تعبير RNA للعديد من جينات التي تسمح لدخول فايروس SARS-Cov2 الإنزيم المحول لأنجيوتنسين 2 (ACE2) ، والبروتيناز الغشائي (TMPRSS2) Serine 2 ، و Cathepsin I في رئة فئران التجارب.

طريقة البحث: تم تقسيم الفئران المخبرية إلى ثمانية وعشرين فأراً من نوع balb/c إلى أربع مجموعات. عولجت المجموعات الثلاث الأولى (جميع الفئران في هذه المجموعات هم ذكور) بعقاقير التستوستيرون ، والأوكساندرون على التوالي ، بينما تألفت المجموعة الرابعة من فئران إناث من غير اعطاء أي عقاقير. تم إعطاء الأدوية للمجموعة الثانية و الثالثة لمدة 21 يوماً بجرعات مكافئة لجرعة الإنسان. بعد ذلك، تم قياس تعبيرات جينات ACE2 و Tmpress2 و CatL باستخدام تقنية real-time PCR. بالإضافة إلى ذلك ، تم تحليل التغيرات النسيجية المرضية في رئة الفئران ومستويات الهرمون الذكري التستوستيرون في دم الفئران.

النتائج: وجدنا في هذه الدراسة أن التعبير الجيني ل ACE2 قد زاد بشكل كبير في رئة الفئران التي تم علاجها بهرمون التستوستيرون بمقدار 2.5 ضعفاً. أيضاً، زاد التعبير الجيني ل TMPRSS2 بشكل كبير في رئة الفئران المعالجة بعقار الأوكساندرون بمقدار 6.6 ضعفاً. إضافة إلى ذلك ، ارتبطت هذه التغيرات الجزيئية بارتفاع كبير لهرمون التستوستيرون في الدم وزيادة الالتهاب والإجهاد التأكسدي في الرئة حسب التحليل النسيجي. إضافة إلى ذلك ، وجدنا أن مستويات RNA لكل من الجينات ACE2 و TMPRSS2 و CatL كانت أعلى بشكل ملحوظ في رئة إناث الفئران مقارنة بالذكور.

الاستنتاج: وجدنا عدة فروق ذات دلالة إحصائية بين التعبير الجيني لجينات ACE2 و TMPRSS2 و CatL في رئة ذكور وإناث الفئران. وجدنا في هذه الدراسة أن إعطاء الهرمونات الذكورية التستوستيرون والأوكساندرون لذكور الفئران يزيد التعبير الجيني ل ACE2 و TMPRSS2 في رئة الفئران. يمكن لهذه النتائج أن تزيد من فهمنا لتأثير الجنس والعقاقير المحتوية على الهرمونات الذكورية على التعبير الجيني لجينات تلعب دوراً أساسياً في دخول فايروس SARS-Cov2 إلى خلايا الرئة.

الكلمات الدالة: رئة، ACE2، أوكساندرون، تستسترون، SARS-Cov2، TMPRSS2، كوفيد-19.