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WHEN OFF TARGET EFFECTS ARE ON TARGET: THE ROLE OF SPIRONOLACTONE IN PATIENTS WITH COVID-19

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Short Title: Spironolactone off target effects

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ABSTRACT

Aims: Spironolactone is a steroidal mineralocoricosteroid receptor antagonist (MRA) used for treatment of resistant hypertension, heart failure and edema. It exerts class specific adverse effects that are shared by other MRAs. Additionally, it exerts unique "off target" steroidal effects that include gynecomastia, impotence and loss of libido in males and menstrual irregularity in females. Together, these have led to a poor tolerability and limited use despite positive results in many randomized, controlled clinical trials. We review the off-target effects of spironolactone that may summate with its MRA action to provide an advantageous profile for prevention or treatment of patients with COVID-19. **Methods:** Literature review using PubMed Central. **Results:** The blockade by spironolactone of the androgen receptor should diminish the expression of transmembrane protease serine 2 (TMPRSS2) that has an androgen promoter while its MRA action should enhance the expression of protease nexin1 (PN1) that inhibits furin and plasmin. TMPRSS2, furin and plasmin cooperated to process the SARS-CoV-2 spike protein to increase its high affinity binding to the angiotensin converting enzyme 2 (ACE2) and thereby promote viral cell entry. Its actions as an MRA may reduce inflammation and preserve pulmonary, cardiac and vascular functions. Its anti-plasmin action may combat hemostatic dysfunction. **Conclusion:** The hypothesis that the off-target effects of spironolactone summate with its MRA actions to provide special benefits for COVID-19 is worthy of direct investigation and clinical trial.

Keywords: mineralocorticosteroid receptor antagonist (MRA); angiotensin converting enzyme 2 (ACE2); SARS-CoV-2; transmembrane protease receptor serine 2; furin; plasmin

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Introduction: Most "off-target" drug effects are detrimental. In this hypothesis review, we consider the off-target effects of spironolactone that may become an advantage if used to prevent or limit the severity of the SARS-CoV-2 virus infection. We review the steroidal "off-target" and the mineralocorticoid receptor antagonist (MRA) "on-target" actions of spironolactone and their potential impact on COVID-19 disease.

Spironolactone was used initially as a diuretic but became established as a drug of choice to treat drug resistant hypertension and has earned a class 1 indication for the treatment of heart failure with reduced ejection fraction (HFrEF) in both US and European guidelines based on the results of the Optimum Treatment for Drug Resistant Hypertension (PATHWAY-2) (1), Randomized Aldactone Evaluation Study (RALES) (2), Eplerenone Post-Acute Myocardial Infarction Study (EPHESUS) (3) and Eplerenone in Patients with Systolic Heart Failure (EMPHASIS-HF) (4) trials. Despite these recommendations, the use of spironolactone has been lessened because of reduced tolerability that derives from a combination of adverse MRA class effects and off-target effects that are specific for spironolactone.

Recent interest has been expressed in a novel use for spironolactone to prevent, or moderate, the symptoms, of COVID-19 first by others (5-7) and by us (8). Moreover, several clinical trials of spironolactone in COVID-19 are planned, or have begun recruitment: Losartan and Spironolactone Treatment for ICU patients with COVID-19 suffering from ARDS (COVIDANCE: NTC 04643691; Bromhexine and Spironolactone for CoronavirUs Infection Requiring HospiTalization (BISCUIT): NTC04345887; Spironolactone in COVID-19 Induced ARDS: NTC04345887). Spironolactone exerts both MRA and the anti-androgen actions that may contribute to protection from COVID-19 (9). Indeed, a small observational trial has reported a significantly reduced rate of admission of COVID-19 infected men taking anti-androgen

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medications (primarily 5- α-reductase inhibitors for prostate cancer) to the intensive care unit (10) while a population-based retrospective study among 4532 male patients from Italy reported a significant 4-fold protection among prostate cancer patients receiving androgen-depriving therapies (11). We present a hypothesis for the relative potential efficacy of spironolactone exerted by its class vs off-target actions, and the implications for its use to prevent or lessen the severity of COVID-19 infection.

Class effects of spironolactone: The major adverse class effect is hyperkalemia that can be a serious, and potentially fatal, complication. It is reported in 19% of patients over the first year of spironolactone therapy and leads to withdrawal of spironolactone in one half of these (12) but is rarely a problem in patients with normal renal function. Hyperkalemia can often be managed with patiromer or zirconium sodium. MRAs also can cause a hemodynamic reduction in the glomerular filtration rate (GFR) but over the long term they may slow the decline in GFR.

Off-target effects of spironolactone: The major off-target effects are breast pain, gynecomastia, impotence and loss of libido in males and menstrual irregularities in females. Spironolactone also can release adrenocorticotropic hormone (ACTH) and raise plasma cortisol (13). The steroidal off-target effects of spironolactone include anti-androgen, pro-progestin and pro-glucocorticoid actions, although the former predominate (14). These are time- and dose-dependent. Anti-androgen effects are encountered in more than half of the patients receiving spironolactone at a high dosage of 100 mg for more than three months (15). The off-target actions have motivated the development of MRAs with lesser effects on serum potassium or more specific binding to the MR and thereby absence of off-target actions.

Differences between MRAs: Eplerenone is a second generation steroidal MRA that lacks the off-target effects of spironolactone (14, 16, 17). Third generation non-steroidal MRAs

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under development include finerenone, esaxerenone, aparenone and KBP-5074. These lack steroidal off-target effects and may also cause less hyperkalemia (18).

Mechanisms of the off-target effects of spironolactone: High concentrations of spironolactone, but not eplerenone, can interrupt steroidogenesis (19). Spironolactone may increase plasma progesterone (20) that has anti-androgenic activity in men. Despite these reports, even 10 months of administration of 200 mg daily of spironolactone that induced gynecomastia in 62% of the male recipients, did not change plasma levels of testosterone, estradiol or progesterone (21). On balance, it is probable that the anti-androgenic activity of spironolactone is not due primarily to inhibition of testosterone generation.

There is stronger evidence that spironolactone inhibits the androgen receptor (AR). Thus, spironolactone and its metabolite canreonate inhibit the binding of radio- labelled 5-alpha-dihydrotestosterone to the cytosolic and nuclear AR in the prostate gland of the rat, where the affinity of spironolactone is 5-fold higher than canreonate (22). Inhibition of the AR by spironolactone is used to reduce androgenic effects in women with polycystic ovarian syndrome and hirsutism.

Spironolactone possesses only weak progesterogenic and anti-estrogenic activity but can regulate the subcellular distribution of estrogen receptors that could underlie its effects on intra-menstrual bleeding and can inhibit 17-beta-hydrosteroid dehydrogenase type 2 that can increase estradiol (23).

Circulating levels of renin, angiotensin II (Ang II) and aldosterone in COVID-19 infected subjects: Presently lacking are serial measurements of the levels of renin, Ang II or aldosterone in patients infected with COVID-19 to provide additional rationale for use of renin-

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angiotensin-aldosterone system inhibitors (RAASi). However, a recent study of patients infected with SARS-CoV-2 reported a 45% incidence of hypokalemia with increased urinary potassium and systemic alkalosis, suggesting hyperaldosteronism (24).

A study of COVID-19 infected patients hospitalized with pneumonia reported a 3-fold increase in circulating levels of Ang II (25) while a similar study reported a 3-fold increase in circulating levels of renin and aldosterone in those severely affected (26). In contrast, studies in less severely affected patients in the US reported normal values for plasma Ang II and serum aldosterone (27). Apparently, the levels of RAAS components can be increased substantially during severe COVID-19 infection. Of interest, plasma levels of cortisol and ACTH were numerically (but not significantly) *reduced* in severely compromised, compared to mild/moderately compromised, patients with COVID-19 (26). Thus, the expected response of activation of the hypothalamic-pituitary-adrenal response by the stress of a severe illness may be suboptimal in COVID-19 infected patients. A metanalysis of dexamethasone use in COVID-19 patients with ARDS concluded that high dose steroids reduce mortality by ~30% (28). However, it is not clear whether a modest increase in plasma cortisol, as reported in some studies with spironolactone, could be beneficial but is a potential component of its off-target effects, although clearly not a substitute for the use of high dose glucocorticosteroids in those developing ARDS.

Pharmacokinetic considerations: Spironolactone has a short half-life of less than 9 hours. It is metabolized principally to canrenone with a longer half-life of up to 58 hours. Adverse anti-androgen effects are less frequent with potassium canreonate than with spironolactone (29). Spironolactone, but not canreonate, downregulates hepatic ARs and upregulates estrogen receptors resulting in a ~ 10-fold greater anti-androgen receptor activity than canrenone (30). Thus, the steroidal anti-androgen effects of spironolactone are mainly a

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property of the drug itself rather than its metabolite, yet both spironolactone and canrenone are potent inhibitors of the MRA.

Dose-dependent and metabolite effects of spironolactone: The Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm trial (ASCOT-BPLA) randomized participants to a mean daily dose of spironolactone of 25 mg for 2.9 years. There was a significant fall in systolic blood pressure and a 6% rate of gynecomastia or breast discomfort in men (31) demonstrating a modest frequency of anti-androgen effects with relatively low doses of spironolactone even over several years. The anti-hypertensive efficacy of spironolactone increased from daily doses of 25 mg to 75 to 100 mg , but there was no additional effect above 150 mg. Gynecomastia developed in 7% of men receiving daily doses of 50 mg or less, but in 52% of those receiving 150 mg or higher (32). Gynecomastia developed in 30% of normal men given spironolactone at 100 mg daily for 10 months but in 62% of those given 200 mg daily. Thus, both the anti-hypertensive and anti-androgenic actions of spironolactone are dose-dependent up to at least 100-150 mg daily but prolonged daily dose of about 25 mg of spironolactone produce generally useful anti-hypertensive effects and tolerable anti-androgen effects. However, for those requiring robust anti-androgen effects, daily doses of 150-200 mg may be necessary. This requires testing in clinical trials.

Host–defense mechanisms against SARS-CoV-2 infection: angiotensin converting enzyme type 2 (ACE 2): A hypothesis for combined class and off target effects of spironolactone to reduce COVID-19 infectivity or disease severity is outlined in Figure 1. There has been concern that patients using a RAASi might be at increased risk for becoming infected with COVID-19 or developing organ damage, since ACE inhibitors or angiotensin receptor blockers (ARBs) can increase circulating levels of ACE2 (33), one domain of which is as the

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receptor for the binding of the spike protein of the SARS-COVID -2 virus to permit its cellular entry in the respiratory epithelium (34). However, subsequent observational studies have not reported that the use of an ACEi or ARB are not associated with an increased risk of developing or dying from COVID-19, and some have suggested beneficial effects (35). This has been ascribed to the function of the second domain of the ACE2 enzyme that metabolizes angiotensin II (Ang II) and generates Ang-1-7 that activates the Mas receptor to reduce inflammation and organ damage (33, 36). Spironolactone given to patients with HF increases the ACE2 levels in circulating monocytes substantially (37), although there are as yet no studies in patients with COVID-19 infection. A reduction in Ang II and an increase in Ang 1-7, may moderate the increases in reactive oxygen species and inflammation that contribute to the mortality of severe COVID-19 infection while an increase in Ang 1-7 in the lungs could counteract the effect of Ang II induced pulmonary dysfunction and fibrosis. Thus, any increase in ACE2 with spironolactone might ameliorate pulmonary injury and organ damage from COVID-19 infection. However, the complex roles of ACE2 as both an essential cell membrane receptor for SARS-CoV-2 and as a potentially protective enzyme, as discussed in great detail (8, 33), suggest that any benefit from increased ACE2 expression may depend on the stage and severity of the SARS-CoV-2 infection and the site of increased ACE2 expression.

Transmembrane protease receptor serine 2 (TMPRSS2): TMPRSS2, furin and plasmin are implicated in the proteolytic processing of the SARS-COV-2 spike proteins that are required for the high affinity binding to ACE2 (8). TMPRSS2 is the essential first step for proteolytic activation of SARS-COV-2 and cleaves both ACE2 and the SARS-S protein (38). It is considered a prime target to combat coronavirus infection (39). TMPRSS2 is expressed modestly in several extra pulmonary sites including human cardiomyocytes (17) where its

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expression is increased with age (40, 41) and male sex (42). As described (8) ACE2 and TMPRSS2 are co-expressed in cells at several strategic sites for SARS-CoV-2 infection including human and mouse conjunctiva, with increased expression in males, bronchial transient secretory cells, pulmonary type II pneumocytic cells, human nasal olfactory epithelial cells and human ileal absorptive enterocytes. Half of pulmonary transient secretory cells co-express ACE2, TMPRSS2, furin and Rho-GTPase that is a signaling molecule for aldosterone (43). Thus, strategies to reduce the expression of TMPRSS2 might reduce SARS-CoV-2 infectivity and bodily spread (8). Indeed, SARS-CoV-2 cellular entry and infectivity can be reduced by protease inhibitors such as nafamostat that inhibit TMPRSS2. This drug also is also an anticoagulant. Although the regulation of TMPRSS2 by the RAAS has yet to be studied, the gene for TMPRSS2 has an AR whose blockade reduces the lethality of SARS-CoV-2 in male mice (44). Thus, the anti-androgen action of spironolactone could reduce the expression of TMPRSS2 in cells that arecritical for SARS-CoV-2 entry and thereby reduce COVID-19 infectivity or complications (45). Therefore, the unique off-target AR blocking activity of spironolactone could be an important benefit in COVID-19 infected patients and thereby place spironolactone as the RAASi of choice for them (8). However, this will require proof from adequately powered, prospective, randomized clinical trials. While the pharmaceutical industry has focused on developing MRAs with greater specificity for the MR, the anti-androgenic of spironolactone could be a unique advantage to reduce TMPRSS2 expression and thereby reduce the risk or severity of COVID-19 infection.

Furin: As described (8), furin is a proprotein convertase that cooperates with aldosterone to enhance the activity of the epithelial sodium channel ($E_{Na}C$) in the renal tubular collecting ducts, pulmonary epithelium, colon, salivary glands, eye and vascular endothelium. Aldosterone

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increases furin activity (46) and regulates alveolar fluid and Na^+ uptake by activation of $E_{Na}C$ where furin cooperates with aldosterone and may contribute to the ability of spironolactone to improve lung diffusion in patients with CHF (47).

Furin is expressed in human nasal epithelial cells and lung. It cleaves the S1/S2 site of the SARS-CoV-2 spike protein to enhance viral binding to ACE2 and its entry into the human lung (48). MRAs are likely to decrease furin since there is an extensive positive interaction between aldosterone and furin in renal tubules and the lung and furin activity is increased by aldosterone (46).

Plasmin: Plasmin is generated in the blood stream from plasminogen whose activity is regulated by tissue plasminogen activator (tPA) and in tissues and body fluids by urokinase (uPA). Plasmin, plasminogen and tPA/uPA generally are elevated in the body fluids of patients with the major COVID-19 risk factors (49). Plasmin may be a driver of complex dynamic interactions of hypercoagulation, leading to extensive microthrombi and organ infarction, combined with widespread anti-coagulant actions from reduced platelets, tissue hemorrhage and increased fibrinolysis termed “hemovascular dysfunction” (50). Plasminogen is expressed in the airways and the alveolar type I and II epithelial cells that are also sites of expression of ACE2 and TMPRSS2 and can cleave the spike protein of SARS-CoV-2 at the furin site, thereby enhancing binding of the virus to ACE2 and viral cell entry (51). Indeed, plasmin has been considered to be a key factor for COVID-19 susceptibility (49). Thus, plasmin and furin may cooperate with TMPRSS2 to modulate host defenses against SARS-COV-2 infection and may enhance the effects of aldosterone to activate $E_{Na}C$ in the renal collecting ducts and pulmonary alveolae. Aldosterone increases plasmin activity (46). This suggests that spironolactone should reduce plasmin, but this has not yet been reported.

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Protease nexin 1 (PN1; serpin E2): PN1 is expressed widely, including in human nasal epithelial cells. It inhibits the activity of many proteases including α-thrombin, plasmin, plasminogen and furin. Aldosterone reduces renal PN1 expression and thereby should increase furin and plasmin activity (46, 52). Thus, PN1 expression is likely to be increased by MRAs, predicting that they will reduce plasmin and furin activity and thereby reduce the proteolytic processing of the spike protein, its binding to ACE2 and virus infectivity. Inhibition of plasminogen/plasmin-induced coagulopathy could be additional benefits of an increase in PN1 with MRAs. However, the effects of MRAs on PN1 have yet to be studied directly.

Additional actions of spironolactone: Whereas a modest immune mediated inflammatory response may assist in resolving infection in patients with COVID-19 infection, some develop “hyper inflammation” or a “cytokine storm” with vasculopathy, thrombotic microangiopathy and intravascular coagulopathy that may underlie ARDS and multi-organ failure (53). The immunothrombosis can entail endothelial cell infection and dysfunction, activation of plasminogen activator inhibitor-1 (PAI-1) and platelets.

MRIs have prominent anti-inflammatory properties. Eplerenone reduces inflammation in a mouse model of viral myocarditis (54). MRAs also modulate “trained immunity”, reduce inflammation and prevent vascular dysfunction in models of inflammation or hypertension (55). Thus, spironolactone could have favorable effects on the oxidative stress, hyper-inflammation and widespread coagulopathy that is reported increasingly in severe COVID-19 infections, but direct clinical trials are lacking.

Conclusion: Whereas the benefit/risk ratio of modulating ACE2 by spironolactone for prevention of COVID-19 is unclear, its “off-target “androgen receptor blocking activity is predicted to reduce TMPRSS2 and should thereby be highly protective. The activities of furin

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and plasmin are predicted to be reduced by MRAs and these effects should be amplified by PN1 whose activity is increased by MRAs. Thus, the anti-androgen actions of spironolactone may combine with its MRA activity to reduce the expression or activity of TMPRSS2, furin and plasmin and thereby reduce the high affinity binding of SARS-CoV-2 to ACE2. Additionally, the effects of MRAs to reduce ENaC activity that should be augmented by reduced furin and plasmin activity, should limit pulmonary edema, improve pulmonary gas exchange , and limit diffuse coagulation and fibrinolysis. These predictions require clinical trials to test their validity but suggest that spironolactone should protect against SARS-CoV-2 infection and also against the pulmonary, cardiac, renal and vascular inflammatory complications. Prospective clinical studies will also be required to determine the optimal dose of spironolactone and the target population. However, the anti-androgen actions of spironolactone might be enhanced by twice daily administration since it has a relatively short plasma half-life and the spironolactone molecule mediates much of the anti-androgen effects, whereas its the major metabolites mediate much of the MRA action and have a much prolonged half-life. Thus, physicians might consider using spironolactone initially twice daily in doses of 12.5 to 25 mg for patients at high risk for SARS-COV-2 infection and in higher doses of 100-200 mg daily in those infected with the virus, providing there are no contraindications such as hyperkalemia.

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FIGURE LEGEND

Figure 1: Hypothesis for class effects (red lettering) and off-target effects (green lettering) of spironolactone to reduce the infectivity and severity of SARS-COVID-2 infection. MRA, mineralocorticosteroid antagonist; ROS, reactive oxygen species; ACE2, angiotensin converting enzyme type 2; PN1, protein nexin 1; ENaC, epithelial sodium channel; ARDS, adult respiratory distress syndrome; TMPRSS2, transmembrane protease serine 2.

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Figure 1: