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Clinical stage molecule PT150 is a modulator of glucocorticoid and androgen receptors with antiviral activity against SARS-CoV-2.

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ABSTRACT

PT150 is a clinical-stage molecule, taken orally, with a strong safety profile having completed Phase 1 and Phase 2 clinical trials for its original use as an antidepressant. It has an active IND for COVID-19. Antiviral activities have been found for PT150 and other members of its class in a variety of virus families; thus, it was now tested against SARS-CoV-2 in human bronchial epithelial lining cells and showed effective 90% inhibitory antiviral concentration (EC90) of 5.55 μM. PT150 is a member of an extended platform of novel glucocorticoid receptor (GR) and androgen receptor (AR) modulating molecules. In vivo, their predominant net effect is one of systemic glucocorticoid antagonism, but they also show direct downregulation of AR and minor GR agonism at the cellular level. We hypothesize that anti-SARS-CoV-2 activity depends in part on this AR downregulation through diminished TMPRSS2 expression and modulation of ACE2 activity. Given that hypercortisolemia is now suggested to be a significant co-factor for COVID-19 progression, we also postulate an additive role for its potent immunomodulatory effects through systemic antagonism of cortisol.

Introduction

PT150, developed initially for treatment of depression (completing Phase 2 clinical trials), is both a down-regulator of androgen receptor (AR) expression [1] and a potent glucocorticoid receptor (GR) antagonist [2,3]. It is one of a suite of related compounds with similar hormonal receptor signaling functionalities that have demonstrated antiviral activity in a number of viruses across many virus families (unpublished data). Some effects are mediated by binding to viral genomic glucocorticoid response elements (GRE's; e.g. in HIV, Ebola, HBV, HCV) [4]. Many viruses without GRE's have also been found to be potently inhibited by members of the platform. Two compounds, PT150 and PT156, were found to have some degree of in vitro antiviral activity against MERS virus. PT150, a clinical stage oral medication with a strong safety profile

[5,6] and an active IND for COVID-19, is now shown to have potency against SARS-CoV-2.

Proposed mechanisms of action for the potential suppression of these viruses by these compounds and, in particular, for treatment of the clinical syndrome of COVID-19 include direct antiviral effects at the virus/cell level and systemic effects arising from the inflammatory modulating activities related to their potent antagonism of cortisol in humans. The direct antiviral effects may relate to AR suppression through subsequent down regulation of transmembrane serine protease 2 (TMPRSS2), an androgen-dependent membrane receptor that primes the SARS-CoV-2 spike protein, and ACE2, the major cell receptor for viral entry [7–10]. Moreover, systemic elevation of circulating cortisol [11] and virus-induced neuro-inflammation [12] are now considered possibly significant co-factors in COVID-19 disease severity and progression. The combination of antiviral

and immune-modulatory effects may provide significant therapeutic value for COVID-19 patients.

Results

Against MERS, in MA-104 cells, PT150 and PT156 both showed an EC50 of 3.1 to 3.2 μM (EC90 not calculated). These were compared to an experimental anti-corona virus compound (M128533) with average EC50 of 0.83 μM. M128533, showed little cytotoxicity by visual or neutral red reads, and CC50 > 100 μM. Both PT150 and 156, however, showed significant cytotoxicity to the MA-104 cells used for the assay: CC50 for PT150 in these cells averaged 3.7 μM and for PT156 averaged 9.2 μM. The reasons for this probably relate, in part, to the significantly different GR structure in this species compared to humans; Vero cells, derived from the same source tissues as MA-104 cells, show no response to dexamethasone [13]. This cytotoxicity made confident assessment of the antiviral efficacy of the compounds difficult.

Since the very high MA-104 cell toxicity is not present in human cells, as demonstrated by the PT compounds' extensive pre-clinical assessments [6,-13–18], the absence of documented tissue toxicities in human clinical trials [6], and, in particular, the absence of toxicity in human epithelial airway cells at a 1 μM dose [3], the subsequent testing of PT150 against SARS-CoV-2 was performed in human bronchial epithelial lining cells. Data for SARS-CoV-2 testing are presented in Table 1. EC90 for the control drug, Remdesivir, was 0.0013 μM. EC90 for PT150 in two parallel replicative experiments were 4.25 μM and 6.80 μM, respectively, averaging 5.55 μM. There were no observed cytotoxicities at the highest PT150 dose given of 30 μM or for the highest dose of Remdesivir (1.66 μM).

Discussion

PT150 and PT156 are two compounds in a novel class of hormone receptor modulators with a primary clinical effect of GR blockade of circulating cortisol, but with low-level GR agonism and AR modulation from its direct binding to the GR receptor [1]. Initial studies against MERS coronavirus in cultured MA-104 cells suggested possible antiviral effects, though cytotoxicity in this vervet kidney derived cell line made confident assessment of actual potency difficult. Based on the possibility of antiviral effects against MERS, PT150 was tested against SARS-CoV-2, though in human bronchial epithelial lining cells to avoid the same cytotoxicity. There was a highly significant SARS-CoV-2 antiviral response with an average EC90 of 5.55 μM (3130 ng/mL).

Data from a previously conducted depression Phase 1 major affective disorder study in humans shows that following a 300 mg oral dose the mean C_{max} concentration was 4100 ng/mL, with a half-life of approximately 10 hours [6]. Clinical dosing has been safely obtained up to 900 mg oral dose per day. These data suggest that a therapeutic

Table 1. Antiviral efficacy: EC_{90} for POP test compound PT-150 against SARS-CoV-2.

Test Compounds	Concentration (μM)	[a]Log$_{10}$ CCID$_{50}$ virus per 0.2 mL	[a]Log$_{10}$ CCID$_{50}$ virus per 0.2 mL	[b]EC$_{90}$ (μM)
PT-150	30	3.00	3.00	5.55
	10	3.00	3.30	
	3	4.00	4.30	
	1	4.67	4.50	
Remdesivir	1.66	2.00		0.0013
	0.17	2.50		
	0.017	3.30		
	0.002	3.67		
Virus Control		4.30		
		4.67		
		5.00		

Each well was scored positive for virus if any CPE was observed as compared with the uninfected control. Vero 76 cells were scored on day 5 and confirmed on days 6 & 7.
[a]Titer results from the virus yield reduction assay.
[b]EC$_{90}$ = 90% effective concentration (concentration to reduce virus yield by 1 log$_{10}$) determined by regression analysis.

concentration should be obtainable by daily oral dosing in an acceptably safe regimen.

No cytotoxicity was seen at the highest PT150 dose given of 30 μM. However, higher dosing would be necessary to specify if an actual CC50, if any cytotoxicity can be identified. As already noted, no significant cytotoxicities were identified in the compound's extensive pre-clinical and clinical (phase 1, 2) studies [6,13–18].

The surprising potency of Remdesivir in this model system requires further exploration. In one other report we have been able to identify of Remdesivir in human epithelial airway cells, it was also found to have significantly higher potency compared to culture systems using Vero and Calu-3 cells [19]. Thus, it seems that potency determinations are highly model-dependent and comparisons between models may not precisely predict actual potency in living animal or human systems.

The antiviral effects of PT150 observed in this study may be related to its capacity for AR antagonism. SARS-CoV-2 infection is mediated in part by cellular expression of TMPRSS2 [6], a molecule that is primarily regulated by AR expression [20,21]. The initiating steps for SARS-CoV-2 depend on priming of its spike protein by TMPRSS2 cleavage, which is therefore considered essential for viral spread and pathogenesis [7]. Hoffman et al have recently provided confirmation that a protease inhibitor targeting TMPRSS2 neutralizes viral entry and that SARS-CoV-2 also depends on ACE2 as its receptor for cell entry [6]. Modulation of ACE2 expression and function by AR, directly by AR regulation of ACE2 expression itself [22] and/or indirectly by TMPRSS2 cleavage of ACE2, may further augment viral entry [23]. Thus, PT150 activity against SARS-CoV-2 may be a result of AR/GR downregulation through inhibition of TMPRSS2 expression and diminished ACE2 activity.

The GR modulating effects of PT150 may also be of clinical benefit. The original clinical trials for major affective disorder showed that patients who showed signs of dysregulated cortisol, with elevated serum cortisol levels, most benefited from the drug [6]. Recent data indicate the important role that upregulation of cortisol is likely to play in progression of COVID-19 to severe disease [11]. The potential importance of cortisol had already been signaled by clinical aspects of patient populations at particular risk for SARS-CoV-2 infection and severe COVID-19 disease; individuals with advancing age [24], obesity and metabolic syndrome [25], and cardiovascular disease [26] also have strong associations for hypercortisolemia [27]. Of course, hypercortisolemia leads to immunosuppression, another risk factor for COVID-19 that is confirmed by the increased risk for COVID-19 in patients maintained on immunosuppression for solid organ transplants [28]. The viral infection itself may lead to hypercortisolemia and resultant immunosuppression, as was seen for SARS [29]. Finally, corticosteroid immunosuppressive therapies for COVID-19 have been associated with poorer outcomes [30]. We therefore speculate that in the early stages of infection, cortisol-

induced relative immunosuppression in COVID-19 patients could inhibit host protective immune responses and potentiate viral infection. Additionally, PT150 and related compounds have demonstrated potent anti-neuroinflammatory properties (personal communication, Dr. Ronald Tjalkens, Colorado State University) which may further impact on systemic progression and outcomes of COVID-19 [31].

This interactivity with immunomodulatory effects of endogenous cortisol raises both opportunity and caution. We postulate potential additive effects between these different mechanisms to reduce vulnerability to infection and to treat COVID-19. Directly blocking viral cell entry, PT150 may be prophylactic for viral infection and inhibitory of viral progression once infected since, as previously noted by Hoffman et al, inhibition of TMPRSS2 (i.e. inhibiting viral spike protein priming) and ACE2 (the viral attachment receptor) is likely to inhibit viral cell entry [7]. Once infected, the indirect systemic immunomodulatory effects of the compound may further inhibit progression and reduce severity of disease, particularly in the early stages of infection and in non-severe COVID-19. However, during advanced stage, severe COVID-19, characterized by

a cytokine storm and other features of hyperimmunity, use of PT150 would likely be contraindicated in that it would interfere with exogenous therapeutic glucocorticoid administration, recently shown to be beneficial in a study of dexamethsone in advanced stage COVID-19 [32,33].

In view of these considerations, we suggest several potential clinical uses of PT150 in SARS-CoV-2 infection and COVID-19 based on its demonstrated safety profile through extensive Phase 1 and Phase 2 clinical trials for depression and its active IND for use in COVID-19 treatment [6]. These older studies involved pulse treatments with PT150 of 2-weeks on/2 weeks off [6]. This protocol was utilized to minimize the possibility of Addisonian syndrome from the cortisol blockade or a rebound Cushing syndrome upon withdrawal of the compound. Neither syndrome was seen, confirming the safety of the dosing schedule. Given that the natural course of most SARS-CoV-2 infections is 2 weeks, this protocol can be readily adapted for treatment of early, non-severe infection, given the roughly 2-week duration of infection until clearance. It may also be useful for developing prophylaxis regimens for use by essential workers with high risk of viral exposure or other exposed persons.

In conclusion, PT150 is a clinic ready, small molecule with a good safety profile and active IND for COVID-19. The relatively low dose needed for achieving corresponding anti-COVID-19 concentrations demonstrated by these in vitro studies of SARS-CoV-2 viral suppression is in keeping with readily obtainable drug concentration levels from previously tested clinical, daily oral doses. PT150 has unique, possibly additive effects as a direct anti-viral and as a potential modulator of the immune system. For these reasons, PT150 merits further study as a treatment for non-severe COVID-19 and as a prophylactic for viral infection, alone and in combination with other potential antiviral agents, such as cathepsin-L-inhibitors, as recently suggested by Liu et al [34].

Methods

MERS

Compounds
PT150 and PT156 were provided as a solid and stored at 4°C upon arrival. Just before the assay, the test compounds were dissolved in DMSO to 20 mg/mL. Eight half-log, serial dilutions in culture medium Minimum Essential Medium supplemented with 5% FBS and 50 µg/mL gentamicin with a highest test compound concentration of 100 µg/mL.

Cell culture
96-well plates were seeded with MA-104 cells purchased from ATCC, MA-104 Clone 1 (ATCC® CRL-2378.1) and incubated overnight. Seeding was performed at a cell concentration that yielded 80–100% confluent monolayers in each well after overnight incubation. The test media used was the culture medium with 5% FBS and gentamicin.

Virus
MERS strain EMC was passaged twice in MA-104 cells to produce the stock. Virus was diluted in the test medium with 5% FBS and gentamicin. The multiplicity of infection (MOI) was 0.001 CCID50 per cell.

Experimental design
100 µL of each concentration were added to 5 test wells on the 96-well plate. Three wells of each dilution were treated with the test virus in test medium. Test medium with no virus was added to 2 wells as uninfected toxicity controls. Six wells were untreated virus controls. The protease inhibitor M128533 (E128533; Epicept Corp, San Diego CA), a known compound with activity against MERS, was assayed in parallel [35].

The plates were incubated at 37°C + 5% CO2 until cytopathic effect (CPE) was apparent in the virus control wells. After CPE was observed microscopically, wells were stained with 0.011% neutral red dye for approximately 2 hours. Dye was then siphoned off and 200 µL 50:50 Sorensen citrate buffer/ethanol for was added for >30 min., agitated, and read on a spectrophotometer at

540 nm. Optical density was converted to a percent normalizing to the cell control wells. EC50 and CC50 were calculated by regression analysis.

SARS-CoV-2

Compounds

The test compound was provided as a solid and stored at 4°C upon arrival. Just before the assay, the compound was dissolved in 100% DMSO at a concentration of 20 mg/mL and further diluted to the test dilutions in the MatTek culture medium (AIR-100-MM).

Cell culture

Differentiated normal human bronchial epithelial (dNHBE) cells were made to order by MatTek Corporation (Ashland, MA) and arrived in kits with either 12- or 24-well inserts each. dNHBE cells were grown on 6 mm mesh disks in transwell inserts. During transportation the tissues were stabilized on a sheet of agarose, which was removed upon receipt. One insert was estimated to consist of approximately 1.2×10^6 cells. Kits of cell inserts (EpiAirwayTM AIR-100, AIR-112) originated from a single donor, # 9831, a 23-year-old, healthy, nonsmoking, Caucasian male. The cells have unique properties in forming layers, the apical side of which is exposed only to air and that creates a mucin layer. Upon arrival, the cell transwell inserts were immediately transferred to individual wells of a 6-well plate according to manufacturer's instructions, and 1 mL of MatTek's proprietary culture medium (AIR-100-MM) was added to the basolateral side, whereas the apical side was exposed to a humidified 5% CO_2 environment. Cells were cultured at 37°C for one day before the start of the experiment. After the 24 h equilibration period, the mucin layer, secreted from the apical side of the cells, was removed by washing with 400 μL pre-warmed 30 mM HEPES-buffered saline solution 3X. Culture medium was replenished following the wash steps.

Viruses

SARS-CoV-2 strain USA-WA1/2020 was passaged twice in vervet kidney cell-derived Vero 76 cells to create the virus stock. Virus was diluted in AIR-100-MM medium before infection, yielding a multiplicity of infection (MOI) of approximately 0.003 CCID50 per cell.

Experimental design

Each compound treatment (120 μL) and virus (120 μL) was applied to the apical side, and compound treatment only was applied to the basal side (1 mL), for a 2 h incubation. As a virus control, 3 of the cell wells were treated with placebo (cell culture medium only). Following the 2 h infection, the apical medium was removed, and the basal side was replaced with fresh compound or medium. The cells were maintained at the air-liquid interface. On day 5, the medium was removed and discarded from the basal side. Virus released into the apical compartment of the tissues was harvested by the addition of 400 μL of culture medium that was pre-warmed at 37°C. The contents were incubated for 30 min, mixed well, collected, thoroughly vortexed and plated on Vero 76 cells for virus yield reduction (VYR) titration. Triplicate and singlet wells were used for virus control and cell controls, respectively.

Determination of virus titers from each treated cell culture

Vero 76 cells were seeded in 96-well plates and grown overnight (37°C) to 90% confluence. Samples containing virus were diluted in 10-fold increments in infection medium and 200 μL of each dilution transferred into respective wells of a 96-well microtiter plate. Four microwells were used for each dilution to determine 50% viral endpoints. After 5 days of incubation, each well was scored positive for virus if any CPE was observed as compared with the uninfected control, and counts were confirmed for endpoint on days 6 and 7. The virus dose that was able to infect 50% of the cell cultures (CCID50 per 0.1 mL) was calculated by the Reed-Muench method [36].

The day 5 values are reported. Untreated, uninfected cells were used as the cell controls.

Author contributions

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Disclosure statement

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