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Inhibition of the replication of SARS-CoV-2 in human cells by the FDA-approved drug chlorpromazine

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ABSTRACT

Introduction: Urgent action is needed to fight the ongoing coronavirus disease 2019 (COVID-19) pandemic by reducing the number of infected cases, contagiousness and severity. Chlorpromazine (CPZ), an antipsychotic from the phenothiazine group, is known to inhibit clathrin-mediated endocytosis and has antiviral activity against severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1) and Middle East respiratory syndrome coronavirus. The aim of this in-vitro study was to test CPZ against SARS-CoV-2 in monkey and human cells.

Materials and methods: Monkey VeroE6 cells and human alveolar basal epithelial A549-ACE2 cells were infected with SARS-CoV-2 in the presence of various concentrations of CPZ. Supernatants were harvested at day 2 and analysed by quantitative reverse transcription polymerase chain reaction (RT-qPCR) for the presence of SARS-CoV-2 RNA. Cell viability was assessed in non-infected cells.

Results: CPZ was found to have antiviral activity against SARS-CoV-2 in monkey VeroE6 cells, with a half maximal inhibitory concentration (IC_{50}) of 8.2 µM, half maximal cytotoxic concentration (CC_{50}) of 13.5 µM, and selectivity index (SI) of 1.65. In human A549-ACE2 cells, CPZ was also found to have anti-SARS-CoV-2 activity, with IC_{50} of 11.3 µM, CC_{50} of 23.1 µM and SI of 2.04.

Discussion: Although the measured SI values are low, the IC_{50} values measured *in vitro* may translate to CPZ dosages used in routine clinical practice because of the high biodistribution of CPZ in lungs and saliva. Also, the distribution of CPZ in brain could be of interest for treating or preventing neurological and psychiatric forms of COVID-19.

Conclusions: These preclinical findings support clinical investigation of the repurposing of CPZ, a drug with mild side effects, in the treatment of patients with COVID-19.

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1. Introduction

From the beginning of the coronavirus disease 2019 (COVID-2019) outbreak, a higher prevalence of symptomatic and severe forms of COVID-19 was observed at Sainte-Anne Hospital (GHU Paris Psychiatrie & Neurosciences, Paris, France) among healthcare professionals (~14%) compared with patients on psychiatric wards (~4%) [1]. This unexpected finding [i.e. that patients with more comorbidities and risk factors (e.g. overweight, cardiovascular disorders, etc.) seem to be protected against symptomatic and severe forms of COVID-19] led to investigate putative factors that could mediate this protection against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). As patients in psychiatric wards receive psychotropic medications, the literature was searched for antipsychotic drugs with antiviral effects.

This literature search identified chlorpromazine (CPZ), an antipsychotic from the phenothiazine group, as the lead candidate [1] for multiple reasons. Firstly, in addition to its antipsychotic activity, CPZ has been used for decades in virology. In-vitro studies have demonstrated that CPZ has antiviral properties against, for example, influenza [2], hepatitis viruses [3], alphaviruses [4], John Cunningham virus [5], Japanese encephalitis virus [6], bronchitis virus [7], MHV-2 [8], Zika virus [9] and dengue virus [10]. In addition, CPZ is the leading drug inhibiting clathrin-mediated endocytosis [11–14] – via translocation of clathrin and AP2 from the cell surface to intracellular endosomes [12] – and therefore is commonly used to determine the pathways of entry of viruses into cells [11–14]. A recent review article underlined the therapeutic potential of targeting clathrin-mediated endocytosis – essential for entry of coronaviruses into cells [15] – to tackle SARS-CoV-2 [16].

Secondly, CPZ has been shown to have antiviral activity against coronaviruses in multiple studies [17–20]. It was identified as active against both Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-1 in a screen of 348 drugs approved by the US Food and Drug Administration (FDA), together with three other compounds (chloroquine, loperamide, lopinavir), using different cell lines [17]. Similar results were obtained in a different library screen [18], as well as in another study using primary human monocytes [19]. More recently, CPZ (and 16 other compounds) has been shown to have antiviral activity against SARS-CoV-2 in monkey Vero E6 cells [20].

As such, the aim of this study was to investigate in-vitro CPZ antiviral activity against SARS-CoV-2 in monkey Vero E6 cells and – for the first time – human alveolar basal epithelial cells.

2. Materials and methods

2.1. Cell culture and virus isolates

Vero E6 cells (African green monkey kidney epithelial cells, ATCC, CRL-1586) were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 5 units/mL penicillin and 5 μg/mL streptomycin at 37°C with 5% CO_2. A549-ACE2 cells (adenocarcinomic human alveolar basal epithelial cells, transduced to express human angiotensin-converting enzyme 2; kind gift from Pr. O. Schwartz, Institut Pasteur, Paris France) were maintained in DMEM containing 10% FBS, 5 units/mL penicillin, 5 μg/mL streptomycin and 40 µg/mL blasticidin at 37°C with 5% CO_2.

SARS-CoV-2 (isolate BetaCoV/France/IDF0372/2020 C2) was supplied by the National Reference Centre for Respiratory Viruses (NRC) at Institut Pasteur, Paris, France, headed by Pr. S. Van der Werf. The human sample from which this strain was isolated was provided by Dr X. Lescure and Pr Y. Yazdanpanah from Bichat Hospital, Paris, France. Viral stocks were prepared by propagation in VeroE6 cells in DMEM supplemented with 2% FBS. Viral titres were determined by plaque assay. All experiments involving live SARS-CoV-2 were performed in accordance with the guidelines of Institut Pasteur for Biosafety Level 3 work. All experiments were performed in at least three biologically independent replicates.

2.2. Antiviral activity assay

Cells were seeded into 96-well plates 24 h prior to the experiment. Two hours prior to infection, cell culture supernatant was replaced with media containing 32 µM, 16 µM, 8 µM, 4 µM or 2 µM of CPZ, or the equivalent volume of water (control). For infection, the drug-containing media was removed and replaced with virus inoculum [multiplicity of infection values of 0.1 plaque-forming units (PFU)/cell for VeroE6 cells and 1 for A549-ACE2 cells] for 2 h. The inoculum was then removed and replaced with 100 µL of fresh media (2% FBS) containing CPZ at the indicated concentration or water and incubated for 48 h.

At 48 h, cell supernatant was collected and spun for 5 min at 3000 *g* to remove debris. Toxicity controls were set up in parallel on uninfected cells. RNA was extracted from 50-µL aliquots of supernatant using the Nucleospin 96 virus kit (Macherey-Nagel, Düren, Germany) in accordance with the manufacturer's instructions. Detection of viral genomes was performed by quantitative reverse transcription polymerase chain reaction (RT-qPCR) using the IP4 primer set developed by NRC at Institut Pasteur (described on the World Health Organization's website: https://www.who.int/docs/default-source/coronaviruse/real-time-rt-pcr-assays-for-the-detection-of-sars-cov-2-institut-pasteur-paris.pdf?sfvrsn=3662fcb6_2). RT-qPCR was performed using the Luna Universal One-Step RT-qPCR Kit (New England Biolabs, Ipswich, MA, USA) in a QuantStudio 3 thermocycler (Applied Biosystems, Foster City, CA, USA) with the following cycling conditions: 55°C for 10 min, 95°C for 1 min, and 40 cycles at 95°C for 10 s, followed by 60°C for 1 min. The quantity of viral genomes is expressed as PFU equivalents, and was calculated by using a standard curve with RNA derived from viral stock with a known viral titre.

Cell viability in drug-treated cells was measured using alamarBlue reagent (ThermoFisher, Waltham, MA, USA). Forty-eight hours post treatment, the drug-containing media was removed and replaced with alamarBlue and incubated for 2 h at 37°C. Fluorescence was measured using an Infinite 200 PRO plate reader (Tecan, Männedorf, Switzerland).

Using the same experimental setting, the antiviral activity of remdesivir was tested as a comparator and validator of the experiment.

2.3. Data analysis

Antiviral activity was assayed as a percentage of inhibition of SARS-CoV-2, normalized to the quantity of viral genomes at the lowest concentration of CPZ (i.e. 2 µM) for each of the three independent replicates. Percentage cytotoxicity was calculated relative to untreated cells (0% toxicity). Antiviral activity and cytoxicity data were analysed using GraphPad Prism Version 8.4.2 for MacOS (GraphPad Software, San Diego, CA, USA; www.graphpad.com). Non-linear regressions were performed and the half maximal inhibitory concentration (IC_{50}), 90% maximal inhibitory concentration (IC_{90}), half maximal cytotoxic concentration (CC_{50}) and selectivity index (SI; calculated by dividing CC_{50} by IC_{50}) were calculated from '[agonist] vs. response – variable slope' curves with constraints to remain above 0% and below 100%.

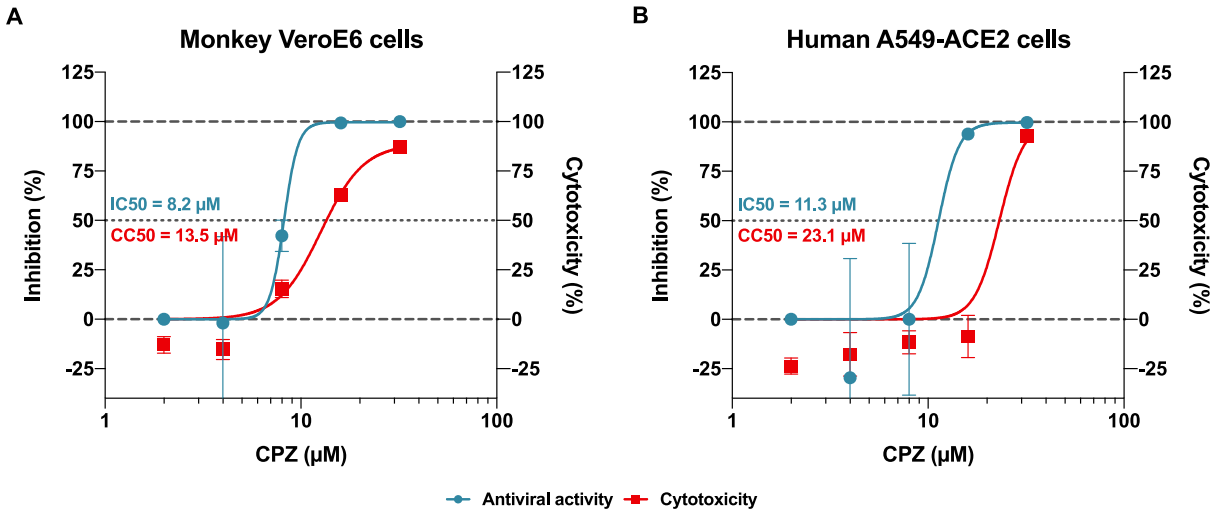

Fig. 1. Antiviral activity of chlorpromazine (CPZ) against severe acute respiratory syndrome coronavirus-2 *in vitro* in monkey VeroE6 cells (A) and human A549-ACE2 cells (B). Viral load in supernatants was measured at 48 h (left Y axis), and viability under increasing concentrations of the antiviral compound is shown. Error bars denote standard error of the mean. IC_{50}, half maximal inhibitory concentration; CC_{50}, half maximal cytotoxic concentration.

3. Results

African green monkey VeroE6 cells and human alveolar basal epithelial A549-ACE2 cells were infected with SARS-CoV-2 in the presence of various concentrations of CPZ. Supernatants were harvested at day 2 and analysed by RT-qPCR for the presence of SARS-CoV-2 RNA. In parallel, cell viability was assessed in non-infected cells.

In monkey VeroE6 cells, CPZ was found to have antiviral activity against SARS-CoV-2, with IC_{50} of 8.2 µM, IC_{90} of 15.2 µM, CC_{50} of 13.5 µM and SI of 1.65 (Fig. 1A).

In human A549-ACE2 cells, CPZ was also associated with anti-SARS-CoV-2 activity, with IC_{50} of 11.3 µM (Fig. 1B) and IC_{90} of 14.3 µM. CPZ had a cytotoxic effect in human A549-ACE2 cells at the highest doses assessed, with CC_{50} of 23.1 µM and SI of 2.04 (Fig. 1B).

Remdesivir was found to have antiviral activity against SARS-CoV-2, with IC_{50} values of 5 µM and 0.15 µM in monkey VeroE6 cells and human A549-ACE2 cells, respectively.

4. Discussion

With more than 6 000 000 infections and 370 000 deaths worldwide in just a few months [21], tools are needed urgently to reduce the severity and contagiousness of COVID-19, and reduce the socio-economic consequences. This study evidenced in-vitro antiviral activity of CPZ against SARS-CoV-2 in monkey and human cells, with IC_{50} values of 8.2 and 11.3 μM, respectively. These results are in line with previous demonstrations of antiviral properties of CPZ, a well-known inhibitor of clathrin-mediated endocytosis [11–14], against other coronaviruses. In addition, the IC_{50} of remdesivir is consistent with previously published work [22,23], reinforcing the validation of the experimental setup.

Although the measured SI values of CPZ are very low, the IC_{50} values measured *in vitro* may translate to CPZ dosages used in routine clinical practice. Indeed, one of the main advantages of using CPZ against SARS-CoV-2 could lie in its biodistribution (Fig. 2), and mainly and foremost in its pneumophilic properties. In 1968, Forrest et al. quantified the distribution of CPZ in selected organs through a post-mortem study of six patients with schizophrenia who were treated with CPZ until death [24]. Among the five patients with lung measurements available, the highest CPZ concentration was found in the lungs. High pneumophilic properties of

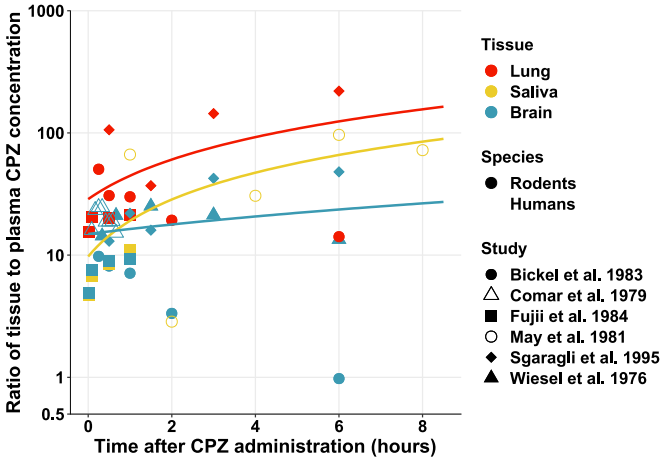

Fig. 2. Review of temporal distribution of chlorpromazine (CPZ) in lungs, saliva and brain. Ratio of tissue to plasma CPZ concentrations (log scale) after administration of a single dose of CPZ are represented for lungs (red), saliva or salivary glands (yellow) and brain (blue) in rodents (solid symbols) and humans (open symbols). Derived from previous preclinical and clinical studies [25–28,31,32].

CPZ have also been described in preclinical studies, reporting a lung concentration of CPZ that is 20–200 times higher than the plasma concentration of CPZ after a single dose [24–27]. Besides the lungs, CPZ concentrations have been demonstrated to be 30–100 times higher in the salivary glands compared with plasma after a single dose of CPZ [26,28]. In humans, May et al. studied the salivary concentration of CPZ in 48 newly admitted patients with schizophrenia, and found concentrations between 1300 and 22 000 ng/mL (i.e. 4.1–69 μM; CPZ molar mass = 318.86 g/mol) 1–8 h after a single dose of CPZ [28]. These high salivary concentrations of CPZ could reduce the contagiousness of COVID-19 [29]. Moreover, because of its lipophilic nature, CPZ crosses the blood–brain barrier [30]. Distribution of CPZ in the brain, underlying its antipsychotic action and side effects, has been described, with a CPZ concentration in brain up to 50 times higher compared with the CPZ concentration in plasma in rodents [25–27,31]. In humans, the distribution of CPZ in the brain was studied in 22 patients with schizophrenia at the study hospital in 1979, with the brain:plasma ratio ranging from 15 to 25 [32]. This could be of interest for treat-

ing or preventing neurological and psychiatric forms of COVID-19 [33], which, to date, have no available therapeutic options. Indeed, remdesivir, the anti-interleukin-6 drug tocilizumab and hydroxychloroquine, three of the most studied drugs in the treatment of COVID-19, do not cross the blood–brain barrier or cross it to a far lesser extent [34–37].

Overall, although the extrapolation from an in-vitro result to a clinically relevant dose is not straightforward, the IC_{50} of 11.3 μM (i.e. 3603 ng/mL) measured *in vitro* in human cells may be compatible with CPZ dosages used in routine clinical practice. Indeed, residual plasma levels of CPZ in patients range from 30 to 300 ng/mL [38], which could correspond to 600–60 000 ng/mL in lungs [24–27] and 900–30 000 ng/mL in saliva [26,28] (Fig. 2). This extrapolation is supported by the observation of a lower prevalence of symptomatic and severe forms of COVID-19 in psychiatric patients.

Repurposing CPZ, a molecule already used in clinical practice, could offer a ready-to-use treatment with well-known and very mild side effects. CPZ has been widely used in clinical practice for the treatment of acute and chronic psychoses for decades. This first antipsychotic medication was discovered in 1952 by Jean Delay and Pierre Deniker at Sainte-Anne Hospital [39]. CPZ has been prescribed for approximately 70 years and has been approved by the US FDA for use in psychiatry and anaesthesiology, with an excellent tolerance profile. CPZ is also used in refractory nausea and vomiting of pregnancy [40], in advanced cancer [41], and to treat refractory headaches in various neurological conditions [42].

5. Conclusions

This first in-vitro study of the antiviral activity of CPZ against SARS-CoV-2 in monkey and human cells supports that CPZ, a well-known drug with antiviral properties and an excellent tolerance profile, could be tested clinically as an alternative to currently used drugs or combinations of drugs for the treatment of COVID-19. This proof of principle for the feasibility of CPZ to treat COVID-19 is a critical step for a future clinical trial (NCT04366739).

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Competing interests: None declared.

Ethical approval: Not required.

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