Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Antiviral Research 178 (2020) 104805

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Ivermectin and COVID-19: A report in *Antiviral Research*, widespread interest, an FDA warning, two letters to the editor and the authors' responses

Caly et al. at Monash University in Australia recently published a paper in *Antiviral Research*, reporting that ivermectin, a medication widely used for the treatment of certain parasitic diseases in humans and livestock animals, inhibits the replication of SARS-CoV-2 in cell culture (Caly et al., 2020). Despite the authors' cautious conclusion that ivermectin "warrants further investigation for possible benefits in humans," the paper has excited widespread interest on medical and veterinary websites, which often incorrectly describe the drug as a treatment or cure for COVID-19. These inappropriate statements led to a warning by the US FDA, that ivermectin in veterinary products should not be used for human therapy,

https://www.fda.gov/animal-veterinary/product-safety-information/fda-letter-stakeholders-do-not-use-ivermectin-intended-animals-treatment-covid-19-humans.

The FDA message also explains that *in vitro* studies such as the report in AVR are "commonly used in the early stages of drug development."

The paper by Caly et al. has also elicited two letters to the editor, which are printed below, followed by the authors' response to both letters. Readers should be aware that neither the letters nor the response has been peer-reviewed, so appropriate caution should be used in quoting or citing them.

Mike Bray, MD
Editor-in-chief
Antiviral Research

Caly L, Druce JD, Catton MG, Jans DA, Wagstaff KM, 2020. The FDA-approved Drug Ivermectin inhibits the replication of SARS-CoV-2 in vitro. Antiviral Res. Apr 3:104787.

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To the Editor

Recently Caly et al. reported *in vitro* activity of ivermectin against SARS-CoV-2 following a single addition to Vero-hSLAM cells, and suggest that these data "demonstrate that ivermectin is worthy of further consideration as a possible SARS-CoV-2 antiviral" (Caly et al., 2020). In isolation, these *in vitro* data are robust and encouraging but the report does not include a correlation of the *in vitro* findings with clinically achievable plasma and, more relevantly, lung concentrations that would permit the determination of whether the macrocyclic lactones (and specifically in this case ivermectin) are genuine therapeutic options.

Caly et al. bathed Vero-hSLAM cells with ivermectin at a concentration of 5μM from 2 hours post-infection SARS-CoV-2 isolate Australia/VIC01/2020 until the conclusion of the experiment. SARS-CoV-2 RNA was determined by RT-PCR at Days 0–3 in both supernatant and cell pellet experiments. The authors noted 93–99.8% reduction in viral RNA for ivermectin versus DMSO control at 24h in supernatant (released virions) and cell associated viral RNA (total virus) respectively. They also describe by 48 hours a ~5000-fold reduction of viral RNA and maintenance of effect at 72 hours. Additional experiments were conducted with serial dilutions of ivermectin to establish the concentration-response profile, and the authors describe ivermectin as a potent inhibitor of SARS-CoV-2, with an IC_{50} determined to be approximately 2 μM under these conditions.

We sought to examine the clinical relevance of the concentrations evaluated in these *in vitro* experiments to those that may be achieved with ivermectin dosing in practice, in order to assist in prioritizing ongoing efforts with finding therapeutics that may be effective in COVID-19.

Ivermectin is one of humanity's most important medicines (Crump and Omura, 2011) and is extensively used for 5 neglected tropical diseases at single oral doses of 150–200 μg/kg, resulting in the mean peak plasma concentrations of approximately 30–47 ng/mL (Merck, 2009). In Phase I studies, doses up to 2000 μg/kg (Guzzo et al., 2002) have been administered in a fasted state or up to 600 μg/kg following a standard high-fat meal. Smit et al. (2019) report that ivermectin 600 μg/kg administered orally resulted in a maximum median concentrations (Cmax) in plasma of 118.9 ng/mL (p5-p95: 45.2–455.1ng/mL), with relatively rapid clearance and a half-life of approximately 3–5 hours.

Similar to Yao et al. who proposed the potential for hydroxychloroquine for treating COVID-19, (Yao et al., 2020) we applied a physiologic-based pharmacokinetic (PBPK) model of ivermectin using the Simcyp platform to explore the plasma and lung concentrations relative to the IC_{50} values against SARS-CoV-2 determined *in vitro*. The ivermectin PBPK model was initially developed to facilitate drug development for parasitic diseases including onchocerciasis and is a full model that allows prediction of tissue drug concentrations. The model has been independently verified. The predicted *versus* observed plasma profiles for ivermectin across clinical studies in the Mectizan NDA were well aligned, Merck (1996) indicating the base model is well defined. Furthermore, the PBPK model was able to predict ivermectin exposures in plasma, adipose and skin to within 1.3-fold of observed data in patients infected with *Onchocerca volvulus* (Baraka et al., 1996)

Simulations were performed using the Simcyp Simulator Version 19 Release 1. Ten virtual trials of 10 subjects aged 18–75 years (50% female) were simulated using the Sim-NEurCaucasion population. In the simulation, high dose ivermectin (600 μg/kg) was administered orally, daily for 3 days and the virtual study

https://doi.org/10.1016/j.antiviral.2020.104805

Available online 21 April 2020

carried on to 9 days. Dosing was in the Fed state and fraction unbound was 0.07 (plasma) and 0.13 (lung). Simulations for mean systemic plasma and lung tissue concentrations are shown in Fig. 1.

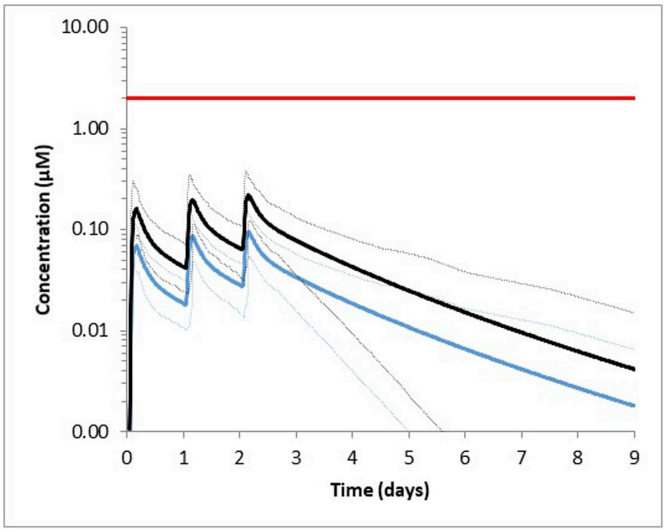

Fig. 1. Simulated mean concentration-time profile of ivermectin in plasma (black line) and lung tissue (blue line) following 600 µg/kg dose daily for 3 days. The 5th and 95th percentiles are also shown. The red-line is the IC_{50} (2µM) against SARS-CoV-2 determined *in vitro* by Caly et al. (2020).

Pharmacodynamic response is generally achieved by ensuring an appropriate duration of exposure above the minimum therapeutic concentration at the site of action. Even with most generous assumptions for clinical translation, the *in vitro* IC50 is > 9-fold and >21-fold higher than the day 3 plasma and lung tissue simulated Cmax respectively, following a high dose ivermectin regimen of 600 µg/kg dose daily for 3 days. (Smit et al., 2019) This dose scenario, which ignores consistent exposure, exceeds the highest regulatory approved dose of ivermectin, being a 200 µg/kg single dose for the treatment of Strongyloidiasis (Merck, 2009).

Caly et al. cite the importance of regulatory approval of ivermectin as a key part of the rationale for further evaluation against SARS-CoV-2. However, the rigorous data review and reassurance of a stringent regulatory authority review only applies to currently approved doses – clinical pharmacology and toxicology margins (including pre-and post-natal and carcinogenicity studies) would, therefore, need to be recalculated. In reality, the resultant unravelling of the supporting package of data could result in lengthy delays while supporting data are revised and re-run.

It is understandable that, faced with a devastating pandemic and a medical and societal imperative, there is great enthusiasm for promising news of treatments. Picking and supporting the best therapies and preventions to tackle the COVID-19 pandemic head on is one of the scientific community's most urgent priorities. To assist this process, the clinical pharmacological relevance of *in vitro* or *in vivo* findings should be included. *In vitro* promise leads to clinical failure in the vast majority of cases, and in the volatile environment of the current pandemic, it is critical that we are sensitive to the implications of our communication and apply our resources to compounds most likely to succeed. A small window exists for the current data to have relevance for humans: we need to confirm the effective concentrations, assess if the class of macrocyclic lactones has similar target interactions, and understand the relevance of the concentrations used *in vitro* against SARS-CoV-2 to those likely to be achieved at the site of action, within a dose range considered to be well tolerated. Alternative routes could also be considered, although these present new formulation and safety challenges. Modeling and simulation approaches integrate *in vitro* findings with the *in vivo* situation and may serve to prioritize existing drugs that are candidates for repurposing.

Declaration of competing interest

KY, DW, LA, MD, PS and CR work for Certara, a consulting firm in integrated drug development and have directly consulted with a variety of not-for-profit global health organizations, biotechnology and pharmaceutical companies and governments with an interest in medical countermeasures against respiratory virus infections. MS works for Medicines Development for Global Health and the Kirby Institute and has no conflicts of interest to declare.

Funding information

No funding was provided to write this short communication.

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To the Editor,

In the context of repositioning/repurposing strategy for urgent unmet medical needs, various drugs are being proposed for the treatment of COVID-19, the pandemic disease caused by SARS-CoV-2 (Noël and Lima, 2020). This is the case for the broad-spectrum macrocyclic lactone ivermectin, as reported by Caly et al. (2020) based on their data showing that ivermectin inhibits the replication of SARS-CoV-2 *in vitro*. However, this *in vitro* activity occurred at much higher concentrations (IC_{50} ≈ 2–3 µM) than the very low (nanomolar) concentrations effective against many nematode species (Geary, 2005), obtained after a usual dose of 200 µg/kg. This micromolar concentration is also higher than the therapeutic peak plasma concentration (about 40 nM) measured in humans treated for onchocerciasis control with a standard dose of 150 µg/kg (Apud Shu et al., 2000) and even after a high daily dose (600 µg/kg) where Cmax of 105–119 ng/ml (0.12–0.14 µM) has been obtained by PK/PD modeling (Smit et al., 2019).

As we previously showed (Pimenta et al., 2010) that ivermectin is a nonselective inhibitor of three important mammalian P-type ATPases (SERCA, Na^+/K^+-ATPase and H^+/K^+-ATPase) at similar micromolar concentrations (IC_{50} ≈ 6–17 µM), we have to be concerned with putative important adverse effects that this

drug could produce at the higher than usual doses that would be necessary for treating COVID-19 patients. As a result, a phase 1 study is absolutely needed before using ivermectin since a recent meta-analysis concluded that there are not enough data to support a recommendation for its use in higher-than-approved doses (Navarro et al., 2020).

Declaration of competing interest

No conflict.

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Response of the authors

To the Editor

Yeo et al. and Noël aptly point out that published studies show that blood levels of ivermectin achieved during standard therapy are much lower than the concentrations we reported as inhibitory for SARS-CoV-2 in cell culture (Caly et al., 2020). Yeo et al. (2020) further explore the question via pharmacokinetic modeling (from Certara Inc.), while Noël and Lima (2020) voices concern that if high concentrations of ivermectin could be achieved, this would likely be toxic. These points are well made, and we are in agreement, but they do not address the reported mechanism of action of ivermectin (Yang et al., 2020), and thereby fail to highlight a further vitally important reason to be very cautious in considering ivermectin as a therapeutic for viral infection in human patients.

Ivermectin's key direct target in mammalian cells is a not a viral component, but a host protein important in intracellular transport (Yang et al., 2020); the fact that it is a host-directed agent (HDA) is almost certainly the basis of its broad-spectrum activity against a number of different RNA viruses *in vitro* (Tay et al., 2013; Yang et al., 2020). The way a HDA can reduce viral load is by inhibiting a key cellular process that the virus hijacks to enhance infection by suppressing the host antiviral response. Reducing viral load by even a modest amount by using a HDA at low dose early in infection can be the key to enabling the body's immune system to begin to mount the full antiviral response before the infection takes control.

Pharmaceutical research efforts are currently underway to refine liquid formulations for intravenous administration of long-acting ivermectin, develop aerosol administration, and consider using ivermectin in combination with other agents to enhance efficacy at low doses. However, it is important to urge great caution in approaching the use of ivermectin in this simplistic way, precisely because ivermectin is a HDA. Because it targets a host component, it cannot be assumed that even doses lower than those discussed by Yeo et al. (2020) and Noël and Lima (2020) are safe in the context of a burgeoning viral infection, where a measured immune response is key to recovery. *Clinical testing of ivermectin at any dose in the fight against viral infection must include intensive monitoring of patient well-being, to pre-empt any immunosuppressive or other adverse reactions as early as possible.*

Finally, it is critically important to remember that *ivermectin as an antiviral is in a very early phase – under no circumstances should self-medication be considered without the guidance of a qualified physician, and especially not using therapeutics designed for veterinary purposes!*

Declaration of competing interest

Authors have no conflict of interest, with no link to any pharma company.

Funding information

No funding supported this letter to the editor.

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