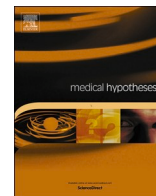




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Inhaled route and anti-inflammatory action of ivermectin: Do they hold promise in fighting against COVID-19?

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ABSTRACT

In an effort to curb the global pandemic due to coronavirus, the scientific community is exploring various treatment strategies with a special emphasis on drug repurposing. Ivermectin, an anti-helminthic drug is also being proposed for treatment and prevention of COVID-19. Ivermectin has demonstrated broad spectrum antiviral activity against both DNA and RNA viruses. Due to its potential to interfere with transport of SARS-CoV-2 nucleocapsid protein to nucleus, it is being proposed to have antiviral activity against this virus as well which has been confirmed in an in-vitro study. However, in-vitro to in-vivo extrapolation studies indicate an inability to achieve the desired IC₅₀ levels of ivermectin after oral administration of doses up to 10 times higher than the approved anti-helminthic dose. In a modelling simulation study, drug accumulation in the lungs was noticed at levels having potential antiviral activity. It is hypothesised that inhaled formulation of ivermectin may be effective against SARS-CoV-2. Therefore, ivermectin administered via inhalational route needs to be explored for potential beneficial role in COVID-19 in preclinical and clinical studies. We also hypothesise the possibility of drug having anti-inflammatory action in coronavirus associated severe respiratory illness based on few in-vitro and in-vivo reports which however needs to be confirmed clinically.

Hypothesis 1: Potential therapeutic role of inhaled ivermectin in covid-19

Background to hypothesis

The COVID-19 pandemic has grabbed the scientific community off-guard globally leaving researchers and clinicians grappling with novel therapeutic strategies in an effort to beat it. At present, there is no proven therapy against this novel deadly virus and the mainstay of treatment is supportive care. Multiple clinical trials are being carried out internationally to come forward with effective treatment options for COVID-19, however the bulk of potential therapeutic modalities are still in the experimental stage. Besides newer investigational strategies, a special emphasis in this direction has been on repurposing the existing time-tested drugs as potential antiviral agents. Drug repurposing in COVID-19 although an ideal strategy yet demands simultaneous establishment of efficacy and safety and at the recommended dose levels.

Ivermectin is an approved broad spectrum anti-helminthic agent which has previously been demonstrated to have anti-viral activity in-vitro against both DNA and RNA viruses including HIV-1, dengue,

West Nile virus, Venezuelan equine encephalitis virus (VEEV) and influenza virus [1]. Ivermectin inhibits the host importin protein $\alpha/\beta 1$ heterodimer involved mainly in the transport of SARS-CoV-2 nucleocapsid protein to nucleus which plays a vital role in viral pathogenesis. As a consequence of this inhibition, the drug is proposed to be beneficial in limiting the disease spread and severity, and has the potential to be used as effective therapeutic and prophylactic agent against SARS-CoV-2 [1]. Caly et al demonstrated the inhibitory role of ivermectin on replication of SARS-CoV-2 in an in-vitro study. In a cell culture model of Vero-hSLAM cells (2 h post infection with SARS-CoV-2), continuous exposure to 5 μ M concentration of ivermectin resulted in approx. 5000-fold (99.8%) reduction in viral RNA load at 48 h; IC₅₀ of ivermectin was determined to be approx. 2 μ M [2]. Publication of this study in early April 2020 generated massive repercussion in social media and huge public and political pressure to legitimize its off-label use in COVID-19. Besides this, other factors like easy availability and affordability of ivermectin, listing in WHO list of essential medicines and long history of proven clinical safety have been the impetus for its repurposing in COVID-19.

Notwithstanding the fact that favourable findings observed in this in

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vitro study give reason for hope, the need to carefully consider benefit-risk profile of ivermectin before embarking on its clinical use can't be denied. In particular, the conviction for possible clinical translation of in vitro findings and repurposing in COVID-19 needs to be meticulously dealt with taking into account the fundamental pharmacological principles. In-vitro to in-vivo extrapolation (IVIVE) demands diligent deliberation of key pharmacokinetic factors. One of the most important factors is the dose required to achieve the desirable IC₅₀ levels in vivo. Even with the administration of doses 10 times higher than approved anti-parasitic dose (200 µg/kg), the maximum plasma concentration is reported to be around 0.3 µM which is far below the desired IC₅₀ values (2 µM). Even with repeat dosing, substantial increase in the plasma exposures could not be observed. [3,4]. In addition, the drug exhibits high protein binding (93%) due to which it has limited endothelial uptake, and hence the IC₅₀ values tend to be even many fold higher than free/unbound plasma levels [5]. Consequently, there is negligible prospect of achieving inhibitory activity of ivermectin against SARS-CoV-2 in humans following oral administration of approved as well as excessive dosing.

Having acquired the knowledge regarding demonstration of antiviral activity of ivermectin in-vitro in the presence of an explained molecular mechanism, do the unfavorable results in IVIVE experiments justify its impractical utility in the management of COVID-19? Being primarily a respiratory virus, is there any avenue for inhalational route of administration of ivermectin in order to achieve desired IC₅₀ levels at the target site of action?

Evaluation of hypothesis

There is no data on the lung tissue disposition of ivermectin in humans. Lifschitz et al determined the lung tissue concentration of the drug in calves (approx. 0.1 µM) after injecting them with 200 µg/kg ivermectin [6]. Similar observations were reported with goats and mice concluding a remote possibility of sufficient accumulation of ivermectin in lungs at conventional doses [7,8]. In a population pharmacokinetic modeling simulation experiment, the researchers predicted an unlikely probability of lung ivermectin concentration attaining the desired IC₅₀ of 2 µM after oral administration of single approved dose (predicted lung concentration: 0.0873 µM) or dose ten times higher (predicted lung concentration: 0.820 µM) [3]. Jermain et al also reported similar results in their physiologically-based pharmacokinetic modelling study [9].

Contrary to these, Arshad et al, by utilizing modelling approach, predicted lung accumulation of ivermectin over 10 times higher than EC₅₀, albeit in the absence of drug's ability to achieve the desired maximum plasma concentrations for antiviral activity (C_{max}/EC₅₀ ratio) [10]. Therefore, likelihood of attainment of higher lung tissue concentrations of ivermectin leaves the door open for further research especially in respiratory infections.

In the light of such supporting evidence, we propose to test inhaled formulation of ivermectin for potential efficacy in COVID-19. Prior to any human exposure, nonetheless, we need substantial evidence in favour of drug's suitability as inhalational agent and its safety and tolerability in animals. As per our knowledge, there is only one published subacute inhalation toxicity study of ivermectin in rats hitherto [11] which reported a no observed adverse effect level (NOAEL) of ivermectin as 380 mg/m². In accordance with USFDA guidelines, non-clinical safety data needs to be generated with a high dose selected to achieve a calculated deposited lung dose of 100 µM (IC₅₀: 2 µM against SARS-CoV-2) and produce a 10-fold margin over the AUC (area under the curve) achieved in humans at the clinical dose [12]. Having proven safety in animal models, inhaled ivermectin can be tested further in clinical trials for potential efficacy in COVID-19. Currently, there are 33 clinical trials registered with clinicaltrials.gov evaluating the role of oral/parenteral/nasal ivermectin in COVID-19 [13]. We hereby advocate to amend the current clinical trial designs to test the hypothesis regarding efficacy of inhaled ivermectin in COVID-19 management.

Hypothesis 2: Ivermectin as anti-inflammatory agent in COVID pneumonia

Background to hypothesis

Rajter et al in their retrospective cohort study (ICON:Ivermectin in COVID Nineteen) reported survival benefit with ivermectin (200 µg/kg orally) compared to usual care in patients with particularly severe pulmonary disease (mortality rate: 38.8% in ivermectin group vs 80.7% in usual care group, Odds ratio: 0.15, CI 0.05–0.47, P = .001) [14]. Although adjustment for few confounders (mortality risks, concomitant drugs etc.) was done by the authors, yet innate biases in observational studies need to be duly scrutinized while concluding their findings. Nevertheless survival benefit observed with oral ivermectin in the presence of remote possibility of antiviral action of the drug at administered oral dose raises speculation regarding another mechanism of drug action which is most likely anti-inflammatory. This is because benefit was significantly more in patients having severe pulmonary disease in which inflammatory changes in the lungs are major hallmark. Hence, it is hypothesised that ivermectin may have an additional anti-inflammatory role in the setting of COVID pneumonia.

Evaluation of hypothesis

Ivermectin has demonstrated anti-inflammatory activity in few in-vitro [15,16] and animal models [17–19]. The mechanism for anti-inflammatory action of ivermectin were explained as inhibition of cytokine production by lipopolysaccharide challenged macrophages, blockade of activation of NF-κB and the stress-activated MAP kinases JNK and p38, and inhibition of toll-like receptor 4 (TLR4) signalling [15–19]. The anti-inflammatory dose was calculated as 18 mg (IVIVE) and 36 mg (allometric scaling) [20].

Molecular interaction of damage-associated high mobility group box 1 (HMGB1) with TLR4 receptor has been identified as a key pathogenetic process leading to lung inflammation associated with SARS-CoV-2 infection [21]. Ivermectin can thus be presumed to exert anti-inflammatory action in SARS-CoV-2 associated respiratory illness. However, whether such a mechanism effectively gets translated in-vivo needs further exploration in preclinical animal models of disease and clinical studies. In this direction, generation of strong evidence particularly rests on appropriately designed and executed randomised clinical trials in patients having respiratory distress and cytokine storm associated with coronavirus disease. Dose finding studies need to be planned to determine the anti-inflammatory dose of ivermectin which is predicted to be higher than recommended clinical dose based on in-vitro results. Also, since ivermectin is metabolised by microsomal enzymes and is a substrate for P-glycoprotein, drug interaction studies need to be carried out with other drugs used for COVID-19.

Conclusion

To conclude, based on existing knowledge scientific rationale for using oral ivermectin in either prophylaxis or treatment of COVID-19 is lacking. So far, expert opinions and few anecdotal reports have been the main driving force for including ivermectin in treatment protocols of COVID-19 across different regions. Ivermectin is being recommended at the antiparasitic dose which is far below the dose assumed to have antiviral action. Visualising the in-vitro to in-vivo extrapolations and simulation experiments, only ray of hope in this direction seems to be inhalational route of the drug which needs vigorous preclinical and clinical testing before being claimed for use in this condition. Also, the clinical potential of drug for use in late stage disease by virtue of its assumed anti-inflammatory action demands further exploration in this direction.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mehy.2020.110364>.

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