The Battle against COVID 19 Pandemic: What we Need to Know Before we "Test Fire" Ivermectin

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ABSTRACT

The world is faced with the dire challenge of finding an effective treatment against the rampaging COVID 19 pandemic. Amidst the crisis, reports of in vitro inhibitory activity of ivermectin, an approved anthelmintic, against the causative SARSCoV2 virus, have generated lot of optimism. In this article, we have fished and compiled the needed information on the drug, that will help readers and prospective investigators in having a quick overview. Though the primordial biological action of the drug is allosteric modulation of helminthic ion channel receptor, its in vitro activity against both RNA and DNA viruses is known for almost a decade. In the past two years, efficacy study in animal models of pseudorabies and zika virus was found to be favourable and unfavourable respectively. Only one clinical study evaluated the drug in dengue virus infection without any clinical efficacy. However, the proposed mechanism of drug action, by inhibiting the importin family of nucleus-cytoplasmic transporters along with favourable pharmacokinetics, warrants exploration of its role in COVID 19 through safely conducted clinical trials. Being an available and affordable drug, enlisted in WHO List of Essential Medicine, and a long track record of clinical safety, the drug is already in clinical trials the world over. As the pandemic continues to ravage human civilisation with unabated intensity, the world eagerly waits for a ray of hope emanating from the outcome of the ongoing trials with ivermectin as well as other drugs.

Introduction

The World is passing through its toughest time after the World War – II . With more than 200 countries or territories affected, over 345 000 confirmed cases and over 2 41000 fatalities as on May 05, 2020, Covid-19 is an "elusive foe" confronting the human civilization. The infection is caused by a single stranded positive sense RNA virus called severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The pandemic has challenged our healthcare system, crippled our economies and jeopardized normal life across the planet. It is encouraging that researchers across the world are

trying their best to tame the pandemic with potential drugs and developed vaccines[1]. Under the umbrella of the World Health Organization (WHO), the SOLIDARITY trial seeks to conglomerate the global data from ongoing randomized controlled trials. A concerted effort is need of the hour to expedite any potential treatment from bench to bedside[2]. Recently, Ivermectin was found to have promising in-vitro inhibitory activity against COVID 19 virus. Using cell lines, Caly L. et al found that a single application of Ivermectin could eliminate 99.98 % of the viral RNA within 48 hours in the 'in vitro' samples. Dramatic reduction in viral RNA in both supernatant as well as in cell associated viral RNA was observed [3]. The drug is already known as a specific inhibitor of nuclear import mediated by Importin alpha/beta – thereby inhibiting replication of RNA viruses [3]. The successful in vitro inhibitory activity of ivermectin against the virus warrants further research to establish its potential role in human COVID 19 infections. In this article, we explored the existing scientific evidence of the drug for possible use in the prophylaxis and treatment of the infection. The compelling information that unravels the potential role of the drug are described under the ensuing sub-sections.

Chemical structure of ivermectin

Ivermectin is a 22,23-dihydro derivative of avermectin B1 from macrocyclic lactone produced by the Streptomyces avermitilis bacterium [4, 5]. Macro cyclic lactones are important precursor compounds in medicinal chemistry. Macrolide antibiotics, antifungal polyene macrolides and some anticancer macrocyclic antibiotics are structural derivatives of macrocyclic lactones [6]. The molecular weight of ivermectin is 870 kDa. The molecule consists of a sugar moiety, a benzofuran head and a spiroketal ring bonded to a central 16-membered macrocyclic lactone ring. Double bonds in the lactone ring form multiple Vander Waals forces. Benzofuran and spiroketal ring are involved in hydrogen bonding while the sugar moiety acts like an inert anchor. The unique chemical structure of macrocyclic lactones enables interaction with a wide array of molecular targets and produces diverse biological actions [5]. It is important to emphasize that Azithromycin, a macrolide antibiotic, has been tended to be useful in treatment of COVID 19 in preliminary studies [7].

Molecular targets of ivermectin

The biological targets of ivermectin have been discovered in viruses, invertebrates and vertebrates. In invertebrates, ivermectin shows affinity towards a number of ion channel receptor family in nerves and muscles. The drug has been widely used as an anthelmintic, pediculicide and insecticide [8]. It also targets several other receptors like Cys-loop receptors, P2X4 receptors and fernesoid X receptors [8]. Theoretically, mammalian ion channel receptors are also targets of the drug. However, at therapeutic concentrations, the affinity of the drug to animal and human receptors are about 100 times less compared to the targeted helminth. Moreover, the Blood-Brain-Barrier prevents the entry of the drug in the central nervous system [5]. Recently, novel mechanisms of anticancer actions of the drug has been identified. It has been shown to promote cell death in cancer cell lines by inducing cytostatic autophagy, caspase-dependent apoptosis and immunogenic cell death through the modulation of specific pathways [9].

In vitro evidence of anti-viral activity

The plethora of biological targets of ivermectin have led researchers to study the antiviral activity of the drug. In in vitro experiments, the drug has been shown to inhibit the growth of a number of viruses. The list of RNA viruses include Human immune-deficiency virus, dengue virus [10], West Nile Virus [11], Venezuelan equine encephalitis virus [12], influenza virus [13] and yellow fever virus [14]. Inhibitory activity has also been shown against DNA viruses-Simian virus SV40 and pseudorabies virus [10, 15]. Amidst the present global crisis struggling with the pandemic, Australian researchers, Caly et al, successfully demonstrated inhibitory activity of the drug in SARS-Cov-2 virus transfected cell lines. The transfected cell lines were treated with serial dilutions of ivermectin (stock = 5μM) at 2 hours after infection. The antiviral activity was assessed by measuring cell-free and cell associated viral RNA load in the samples at 0, 1 and 3 days. The IC50 of the drug with different samples and assay method at 48 hours post infection was found to range from 2.2–2.8 μM. No cytotoxic effect of ivermectin was observed in either the drug treated transfected samples or drug alone non-transfected samples The pioneering work has led to the off-label use of ivermectin at many centers and is an important evidence for ongoing and future clinical trials with the drug. The authors humbly opined "This Brief Report raises the possibility that ivermectin could be a useful antiviral to limit SARS-CoV-2" [3].

Mechanism of antiviral action

Ivermectin is a versatile target binder with selective affinity and inhibitory activity against multiple proteins and enzymes. The primary mechanism is believed to be selective inhibition of host importin α/β transporter protein which decreases translocation of viral nucleocapsid protein (NCP) from the cytoplasm to the nucleus. Altered NCP distribution disrupts viral propagation and survival [12, 16]. It has been shown to reduce nuclear entry of HIV integrase and NS5 polymerase in HIV-1 and dengue virus respectively [10, 11]. In yellow fever virus, it has anti-helicase activity against Non-structural Protein 3 (NS3) decreasing viral RNA replication [14]. The mechanism of antiviral activity in DNA virus is also by inhibition of cytoplasm to nucleus transport of NCP. In cell lines transfected with pseudorabies virus, ivermectin was shown to disrupt the nuclear localization of UL42 NCP by targeting the nuclear localization signal of the protein [15].

Proposed mechanism in COVID 19

Research on earlier SARS CoV to which SARS CoV2 is closely related, shows that the virus possess a well-developed system to interact with the host nuclear import pathway. Sequestration of the viral NCP into the host nucleus through the nuclear-pore-complex (NPC) is a vital step in viral pathogenesis and defence against host immune response. Rapid nuclear localization takes place in the initial period of infection at the time of primary translation, followed by a quiescent stage during which the virus replicates in the cytoplasm. The stored NCP return to the cytoplasm in the later stage of infection to participate in assembly and release. Most transport through the NPC is mediated by members of the importin superfamily, which recognize nuclear localization sequences (NLSs) or nuclear export sequences (NESs) on cargo molecules for transport into and out of the nucleus, respectively [17]. Ivermectin inhibits

host Importin protein Imp α/β1 heterodimer which is the major transporter in nucleocytoplasmic shuttling (NS) of the SARSCoV nucleocapsid protein . It is proposed that ivermectin, by inhibiting NS early in the course of infection, will lead to attenuation of the severity, duration and spread of the infection [3]. The drug has the potential to be used in both prophylaxis and treatment of the feared viral infection.

Evidence in animal models

Studies on the efficacy of ivermectin in animal models of viral infections are few and ambivalent. Based on in vitro evidence, Ketkar et al investigated the role of the drug in prevention of lethal Zika infection in Ifnar1 knockout mice. The results were not favorable. At the normally used dose, the infection and death rate were very similar in the test and control group to be of any significance. The authors found that use of higher dose to achieve the therapeutic concentration predicted by the in vitro experiment proved too toxic for the control group leaving no scope for further investigation [18]. Conversely, in a murine model of pseudo rabies virus infection, ivermectin treatment increased the survival rates of infected mice and also decreased infection severity and gross intracranial lesions [15]. In a rather interesting and unique study involving induced Dengue virus infection in Aedes albopictus mosquitoes, ivermectin fed mosquitoes were found to have significantly lower rate of infection as well as decreased tissue viral load [19]. Ivermectin have also been found to have anti-allergic and anti-inflammatory actions in asthma model of mice. At 2 mg/ kg, the drug significantly suppressed recruitment of immune cells and cytokine production in the bronchoalveolar lavage fluids. It decreased serum levels of ovalbumin-specific IgE and IgG1 and reduced mucus hypersecretion by goblet cells [20]. This may be of added advantage in the treatment of COVID 19 infection where immune-mediated ARDS have been reported [21].

Clinical evidence

Despite the positive results in in vitro experiments, the clinical use of ivermectin in viral infections has been seldom tried in the history of medical science. Renewed interest in the molecule has surfaced in the face of COVID 19 pandemic with a recent acclaimed study reporting its in vitro efficacy [3]. There is mention of a phase III randomized clinical trial on the efficacy and safety of ivermectin in dengue infection in records available with clinicaltrials.gov and a few research articles [3, 22]. Reportedly, there was significant reduction of serum levels of viral NS1 protein without any clinical or virologic benefit [3].

Pharmacological "dissection"

Oral use of ivermectin is approved in humans as an anthelmintic. Like azithromycin, the drug is highly lipophilic, rapidly absorbed (T_{max} = 4 hours), strongly binds to plasma proteins (93 %), undergoes enterohepatic circulation, has propensity for sequestration in tissues (Volume of distribution ≈ 3.5 liters/Kg), resulting in a shorter distribution half-life and a longer terminal half-life (81–91 hours). The drug has, therefore, a prolonged mean residence time in the host associated with persistent effect in vivo [23–25]. After a single oral dose of 150 mcg/kg, the C_{max} and T_{max} are 40 ng/ml and 4 hours respectively. With higher doses (up to 2000 mcg/kg), there is a proportionate predictable increase in Cmax and AUC without any significant change in the number of reported adverse events [24, 26]. The plasma concentration achieved with the maximum tested dose of 2 mg/Kg body weight was around 250 ng/ml. It is reasonably speculated that the projected therapeutic level of 2500 ng/ml for in vivo efficacy in COVID 19, calculated on basis of recent in vitro study, is impractical [22]. However, the prolonged sequestration of the drug in lung tissues at 3 times the plasma concentration in animal studies keeps the prospects open for further research [27, 28]. The IC50 for ivermectin in the in vitro study was determined at 48 hours post infection. Ivermectin treatment to the transfected cell line was given at 2 hours post infection. There is ample ground to continue to explore the pre-clinical research with alteration in observation and experiment timepoints. Intuitively, the drug-receptor binding between ivermectin and Importin protein α/β1 at the host nuclear-pore-complex appears to be a non-competitive/allosteric interaction. Initial transport of importin mediated viral nucleocapsid protein into the nucleus is followed by a quiescent stage during which there is minimal viral growth. After the brief lull, viral replication and release are amplified to uncontrollable proportions. The initial transport acts like a trigger which sets the viral life cycle rolling [17]. Inhibition of the importin receptor prior to infection may dampen the intensity and rate of viral growth. It may also prevent and decrease infection of non-infected host cells. The proposition can be tested in vitro and opens the frontier for evaluation of the drug in clinical studies for prophylaxis as well as treatment.

Clinical safety

The drug has proven record on safety in human use, with the total doses distributed in the last 30 years equalling one-third of the present world population [22]. In a Phase I dose escalating study, the drug was found to be of similar safety and tolerability to placebo even at 10 times the maximum recommended dose [26]. Concerns have been raised about adverse drug-drug interactions (example microsomal enzyme inhibition, Pgp inhibition) when ivermectin will be used concurrently with other drugs (lopinavir/ritonavir) in COVID 19 treatment. However, polypharmacy is a risk which is rampant rather than exclusive and we recommend medication reconciliation and rational prescribing as a routine practice in patient care[29].

Affordability and availability

Ivermectin is enlisted in the core list of WHO Model List of Essential Medicine 2019. The core list incorporates the "minimum medicine needs for a basic health-care system, listing the most efficacious, safe and cost–effective medicines for priority conditions. Priority conditions are selected on the basis of current and estimated future public health relevance, and potential for safe and cost-effective treatment" [30]. The drug is also enlisted in the National List of Essential Medicines of many member nations. It is made available and affordable to the community as part of national health policy of the member countries towards commitment for global health care. The drug is also available commercially at reasonably affordable price in most parts of the world.

Ongoing trials with ivermectin

Globally, as per information available online at clinicaltrials.gov on 4th of May 2020, two health care organizations, one each in India and Iraq, have started recruiting participants in placebo controlled interventional trials. Three other trials are enlisted which are going to start recruitment. The results of the earliest completing trial is expected to come by July 2020 [31].

Vigilant Optimism and Conclusion

In the alarming global situation, we are running out of time and we must act fast, lest the virus dooms the great human civilization. Nevertheless, the drug must pass through rigorous regulatory and ethical checkpoints to safeguard patient vulnerability and scientific standards. In absence of any drug with proven benefit in COVID 19 infection, there is an opportunity to conduct placebo controlled randomized trials. The level of evidence generated with meticulously conducted preliminary trials can unfurl the feasibility of future use of the drug to combat the galloping crisis.

Conflict of Interest

The authors declare that they have no conflict of interest.

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