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BRIEF COMMUNICATION

Ivermectin, antiviral properties and COVID-19: a possible new mechanism of action

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Abstract

Ivermectin is an antiparasitic drug that has shown also an effective pharmacological activity towards various infective agents, including viruses. This paper proposes an alternative mechanism of action for this drug that makes it capable of having an antiviral action, also against the novel coronavirus, in addition to the processes already reported in literature.

Keywords Ivermectin · Antiviral · Ionophore · COVID-19 · SARS-CoV-2

Ivermectin [mixture of 22, 23-dihydroavermectin B1a (80%) and 22, 23-dihydroavermectin B1b (20%)] (Fig. 1) is a macrocyclic lactone with a broad-spectrum antiparasitic pharmacological activity (Gonzalez Canga et al. 2008). It is the safest and most effective semi-synthetic derivative of the entire class of avermectins, discovered in 1975 by Professor Satoshi Ōmura as fermentation products of the actinomycete bacterium *Streptomyces avermitilis* (Crump 2017) (later reclassified in *S. avermectinius* (Takahashi et al. 2002)). Its main pharmacodynamics is to bind some channel proteins for chlorine controlled by glutamate, typical of specific classes of invertebrates, causing a greater permeability to this electrolyte: all this causes a hyperpolarization of the cell membrane, blocking inhibitory neurotransmission in neurons and myocytes, resulting in paralysis and death (Geary 2005). Commercialized since 1981, its low cost, its high efficacy and safety, and the marked tropism for helminths (therefore with an almost zero impact on the biochemistry of human beings) have led to its inclusion in the twenty-first World Health Organization's List of Essential Medicines (World Health Organization 2019).

Ivermectin is a versatile drug with unique characteristics, which make it interesting also for basic and applied research (in particular for drug repurposing): it seems to reveal an antibacterial (Lim et al. 2013; Ashraf et al. 2018), antiviral, and anticancer activity (Juarez et al. 2018; Intuyod et al. 2019), besides being potentially useful for the treatment of some chronic pathologies (Ashraf and Prichard 2016; Ventre et al. 2017), result of an action on a wide range of cellular targets.

Regarding its role as an antiviral agent, its efficacy has been demonstrated on several viruses, both in vitro and in vivo. Among the many mechanisms by which it performs its function, the most consolidated one sees ivermectin as an inhibitor of nuclear transport mediated by the importin α/β1 heterodimer, responsible for the translocation of various viral species proteins (HIV-1, SV40), indispensable for their replication (Wagstaff et al. 2011; Wagstaff et al. 2012). This inhibition appears to affect a considerable number of RNA viruses (Jans et al. 2019; Caly et al. 2012), such as Dengue Virus 1-4 (DENV) (Tay et al. 2013), West Nile Virus (WNV) (Yang et al. 2020), Venezuelan Equine Encephalitis Virus (VEEV) (Lundberg et al. 2013), and Influenza (Gotz et al. 2016). In addition, ivermectin has been shown to be effective against the Pseudorabies virus (PRV, with a DNA-based genome), both in vitro and in vivo (Lv et al. 2018), using the same mechanism. Caly et al. (Caly et al. 2020) have recently shown that the drug also inhibits the replication of the SARS-CoV-2 virus in vitro, however not clarifying how it occurs. Since the causative agent of COVID-19 is an RNA virus, it can be reasonably expected an interference with the same proteins and the same molecular processes described above.

However, ivermectin could prove to be a powerful antiviral, therefore also useful for a possible treatment of the new coronavirus associated syndrome, even from a new perspective. This could happen assuming its role as an ionophore agent, only hinted in the recent past but never fully described (Juarez et al.

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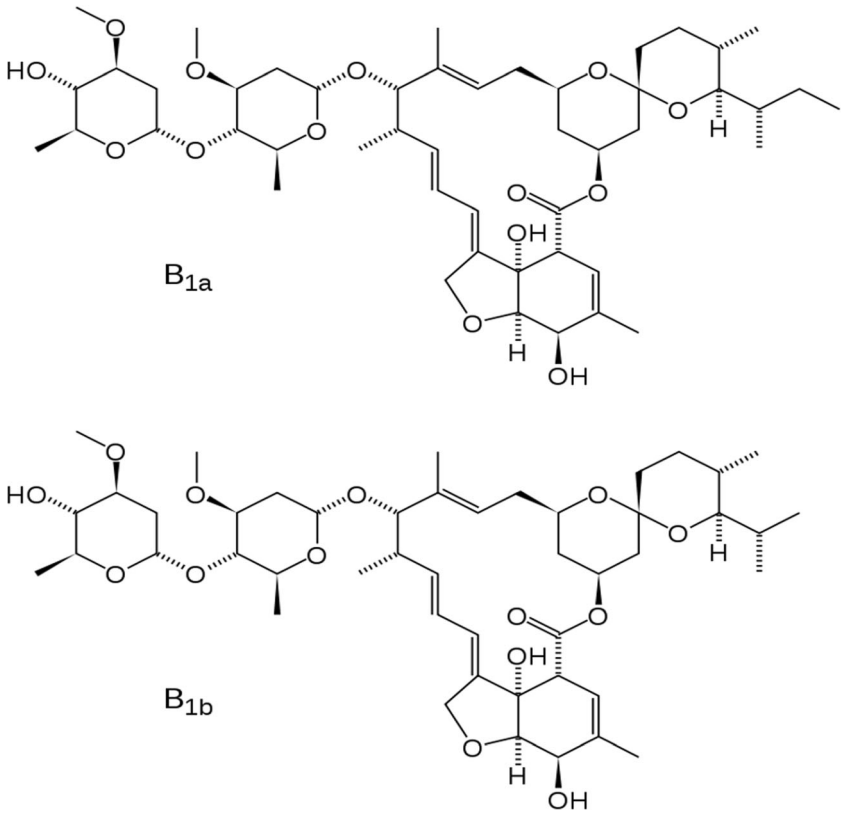

Fig. 1 Structural formulas of ivermectin compounds

2018). Ionophores are molecules that typically have a hydrophilic pocket which constitutes a specific binding site for one or more ions (usually cations), while its external surface is hydrophobic, allowing the complex thus formed to cross the cell membranes, affecting the hydro-electrolyte balance (Freedman 2012). These chemical species have historically been used to study the mitochondrial respiratory chain and ATP synthesis in eukaryotes (in this case also known as decoupling agents, such as 2, 4-dinitrophenol), and their antibiotic activity has long been appreciated (Bakker 1979). It is also hypothesized their role as antiviral drugs (Krenn et al. 2009; Sandler et al. 2020) and anticancer chemotherapeutic agents (Kaushik et al. 2018). Thinking of the structure of two of the most important ionophores, monensin A and valinomycin, respectively a polyether and a depsipeptide antibiotic, it is clear that they internally present many oxygen atoms (with related free electron doublets), indispensable for binding cations and transporting them through phospholipidic bilayers.

At a first glance, the two structures that make up the ivermectin formula do not have these chemical properties, nor those mentioned above, essential for a compound to be defined as ionophore. However, it can be hypothesized that two ivermectin molecules, reacting with each other in a "head-tail" mode, can create a complex suitable to be considered such (Fig. 2). This interaction could occur spontaneously or be mediated by the binding of the same molecules to some plasma transport proteins, in particular albumin (Klotz et al. 1990), which would have the role of positioning them in the correct way to obtain the proposed configuration.

As it can be seen, in this way, an internal cavity is formed: the oxygen atoms (indicated in red), now present in greater number, work as Lewis bases and could therefore coordinate a series of cations (Lewis acids). On the other hand, the –OH groups are highlighted in blue and they could have a decisive role in the stabilization of the new structure, with the establishment of chemical bonds between these functional groups: one or more –O– bridges (however, it is difficult the formation of ether bonds, since acid catalysis at high temperature is not possible under normal conditions, both in vitro and in vivo) or more probably hydrogen bonds could be formed, even among more molecular complexes of this type. However, the formation of other weak and strong interactions of various kinds cannot be excluded. Otherwise, specific cations could bind the two molecules in the proposed way, creating themselves the final structure and stabilizing it: there are examples already known in literature (Abbott et al. 1979). The external part of the complex, then, would already have in itself all the hydrophobic characteristics necessary to carry ions through the viral membrane. As a consequence, it would be determined an ionic imbalance between the external and internal environment, with the recall of water and consequent osmotic lysis. This would allow to neutralize the virus at an early stage of the

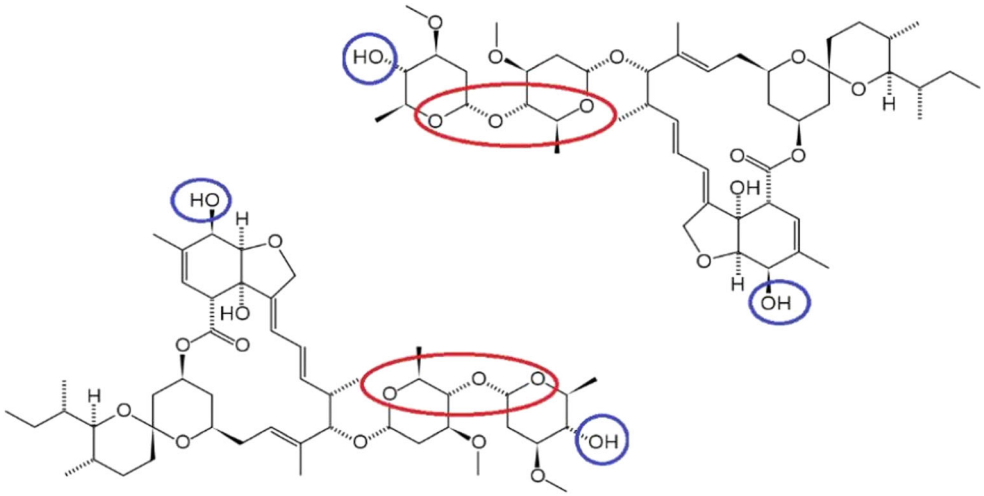

Fig. 2 Possible interaction mechanism between two ivermectin molecules

infection, before; therefore, it can adhere to the host cells and enter it to exploit their biochemical machinery for the production of other viral particles. However, this hypothesis would concern only viruses without a proteic capsid, a structure that shows a certain resistance to osmotic pressure, even if to a lesser extent than a bacterial, fungal, or plant cell wall (Cordova et al. 2003). The new coronavirus is one of these, presenting only a phospholipid envelope in defense of the genetic material, where its few proteins are inserted and which it acquires in the act of exiting the infected cells (Sigrist et al. 2020). This unconventional electrolyte uptake mode could also affect the potential of the viral membrane, threatening its integrity and functionality. The same goes for the viral proteins present here. Furthermore, the concentration variation of some cations, thus determined, could inhibit some key enzymes in the viral replication, such as RNA-dependent RNA polymerases (RdRp) (te Velthuis et al. 2010), already used as pharmacological targets.

Another indication in favor of a possible ionophore role for ivermectin comes from the analysis of molecular similarity that can be carried out through the Drugbank database (www.drugbank.ca). By setting a minimum similarity threshold for ivermectin equal to 0.7, about 14 results are obtained. Among the various selected molecules, the majority of which have antiparasitic and antibiotic activity (already not only on the market but also in the study and experimentation phase), a compound that has high structural similarity is nystatin (score of 0.72), an antimycotic drug with an ionophoric activity at the plasma membrane level, where it forms channels (Yamasaki et al. 2011; Stillwell 2016; Rang 2015).

Immediately afterwards, with a slightly lower similarity, it can be find amphotericin B and natamycin, all pharmacological molecules of assured ionophoric activity (score of 0.71 and 0.706, respectively) (Stillwell 2016; Rang 2015; Ramos 1989; Ikehara et al. 1986).

In conclusion, pending computational simulations and chemical-physical laboratory analysis, this hypothesis could be applied to other known pharmacological molecules, in order to identify compounds with probable ionophore nature to be used in research and clinical practice.

Authors' contributions All research phases (idea, drafting of the paper, and proofreading) were conducted by the only author, ER.

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