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Could the COVID-19-Driven Increased Use of Ivermectin Lead to Incidents of Imbalanced Gut Microbiota and Dysbiosis?

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Abstract
The microfilaricidal anthelmintic drug ivermectin (IVM) has been used since 1988 for treatment of parasitic infections in animals and humans. The discovery of IVM's ability to inactivate the eukaryotic importin α/β1 heterodimer (IMPα/β1), used by some viruses to enter the nucleus of susceptible hosts, led to the suggestion of using the drug to combat SARS-CoV-2 infection. Since IVM has antibacterial properties, prolonged use may affect commensal gut microbiota. In this review, we investigate the antimicrobial properties of IVM, possible mode of activity, and the concern that treatment of individuals diagnosed with COVID-19 may lead to dysbiosis.

Keywords Ivermectin · Gut microbiota · SARS-CoV-2 · COVID-19

Introduction

Ivermectin (IVM), produced by *Streptomyces avermetilis* (previously classified as *S. avermectinius*) [1], is successfully used in the treatment of onchocerciasis (river blindness) caused by the filarial (arthropod-borne) nematode *Onchocerca volvulus* [2, 3]. Since the approval of IVM in 1988 as an antiparasitic drug, 600 million people were treated over little less than two decades [2]. IVM binds to the glutamate-dependent chloride channels of invertebrate nerve and muscle cells, which leads to an increase in membrane permeability and neuromuscular paralysis of certain parasites. The drug is strictly microfilaricidal [4] and as such prevents the development of adult nematodes that cause blindness [2]. Other parasitic infections that have been treated with IVM include ascariasis, cutaneous larva migrans, filariases, gnathostomiasis, lymphatic filariasis, and trichuriasis [5, 6]. Further properties of IVM were demonstrated in the killing of lice and mites associated with pediculosis and scabies [5]. More recent findings have shown that IVM is also effective in killing vectors and parasites associated with malaria (*Anopheles* and *Plasmodium* spp., respectively), fly larvae causing orbital myiasis, roundworms such as *Trichinella* spp. responsible for trichinosis, *Demodex* mites linked with rosacea and cancer cells [7–15].

Further investigation into the bioactive capacities of IVM resulted in the discovery of the drug harbouring antiviral activity, at least in vitro [16–19]. Amongst the first antiviral findings reported was inactivation of the integrase protein of the human immunodeficiency virus-1 (HIV-1) and the importin α/β1 heterodimer (IMPα/β1) that assists the protein with entering the nucleus [20]. The IMPα/β1 heterodimer is an integral trafficker of host proteins [21]. Viruses exploit this heterodimer system to circumvent host immune responses and to enhance the replication of virions [22, 23]. A follow-up study by these authors revealed that IVM could also inhibit the replication of the HIV-1 virus [19]. Despite viral transport into the nucleus by the IMPα/β1 heterodimer being integral to viral infection, small-molecule inhibitors of this heterodimer (such as IVM in this instance) have only had their antiviral activity documented for the past decade [21]. Due to IVM's ability to inhibit nuclear import of host- and viral proteins [24, 25], IVM is able to prevent the replication of the West Nile Virus (flavivirus) [26], yellow fever virus [27], dengue [18], Japanese encephalitis, and tick-borne encephalitis [17, 27]. This is not surprising, as many RNA viruses rely on binding to IMPα/β1 to enter nuclei [28, 29].

Flaviviruses share many similarities with the + ssRNA severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) responsible for the COVID-19 pandemic. Because of

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this, the use of IVM as a prophylactic and treatment against SARS-CoV-2 infection has led to much intrigue and controversy and, hence, a renewed interest in IVM [30–34]. Caly et al. [33] reported a MOI (multiplicity of infection) value of 0.1 for 2 h in Vero/hSLAM cells after exposure to 5.0 µM IVM and suggested that a single dose could control viral replication for 24–48 h. These findings were, however, based on in vitro experiments, and it is difficult to extrapolate to clinical conditions. Schmith et al. [35] argued that 5.0 µM IVM, which resulted in 50% inhibition (IC_{50}; 2 µM), was at least 35 times higher than the maximum plasma concentration (C_{max}), which was determined to be 0.05 µM (46.6 ng/mL) after oral administration of fasting individuals (200 µg/kg bodyweight; FDA approved dosage). The authors argued that the total (bound and unbound) plasma concentration of IVM and unbound levels are below the IC_{50} value, even when administered at levels 10×higher than the FDA-approved dosage. They also argued that a single dosage of IVM, orally administered, does not reach IC_{50} levels in the lungs, not even when administered at doses 10×higher than the approved. Based on these findings, Schmith et al. [35] concluded that it is highly unlikely that clinical trials with IVM would show inhibition of SARS-CoV-2 and that IVM on its own, at recommended concentrations, may thus not be successful in the treatment of COVID-19.

Along with antiviral activity, several reports documenting direct pathogenic bacterial inhibition by IVM have been published [15]. However, literature in this field is sparse, and little is known about the possible mode of action of IVM on microorganisms. This is likely due to the possibility that drug targets for IVM are absent/scarce in microorganisms. The plausibility of this theory is strengthened by the fact that there are no known homologues of IMPα/β1 or glutamate-dependent chloride channels in prokaryotes. However, *Saccharomyces cerevisiae* and other lower eukaryotes such as *Caenorhabditis elegans*, *Drosophila melanogaster*, *Danio rerio*, *Xenopus tropicalis*, *Gallus gallus* and *Mus musculus* have β-karyopherin import receptors making them possibly sensitive to IVM. Despite the apparent absence of IVM targets, antibacterial activity attributed to IVM has been documented and was reported for the first time in 2018 by Ashraf et al. [36].

The question we ought to ask is whether IVM, orally taken, has any effect on gut microbiota and, if so, what long-term effects could emanate from prolonged treatment. This review sketches possible scenarios and addresses some of the concerns related to gut impairment associated with IVM administration.

Antibacterial Activity of IVM

Ashraf et al. [36] showed that IVM acted as a bacteriostatic agent (confirmed by time-kill kinetics assays) against clinical isolates of methicillin-resistant and methicillin-sensitive strains of *Staphylococcus aureus* (MRSA and MSSA, respectively). The authors reported 12.5 µg/mL IVM as minimum inhibitory concentration (MIC) against MRSA, which is double the MIC determined against MSSA (6.25 µg/ml). Tan et al. [37], however, reported bacteriostatic MIC values of 20.0 µg/mL against the methicillin-resistant *S. aureus* ATCC 43,300. The authors have also shown that a derivative of IVM, with an OH group replaced by an NH_2 group at carbon position 4 (referred to as molecule D4), was much more effective against MRSA and reported a fourfold decrease in MIC values.

The natural form of IVM, a semisynthetic mixture of two chemically modified avermectins comprising 80% 22,23-dihydroavermectin-B1a and 20% 22,23-dihydroavermectin-B1b [15], also inhibited the growth of multi- and extensively drug-resistant strains of *Mycobacterium tuberculosis* (MDR-TB and XDR-TB, respectively) [38]. The MIC_{90} of IVM, determined against 33 strains of *M. tuberculosis* (of which seven were classified as XDR-TB), ranged from 1.5 to 16.0 µg/mL [38]. Interestingly, the MIC_{90} of IVM against the seven isolated XDR-TB strains ranged from 3.0 to 12.0 µg/mL, which is low considering their drug resistance. Five of the 33 strains were not affected by IVM. Time-kill kinetic studies with *M. tuberculosis* strains exposed to 20 µg/mL IVM over 21 days resulted in a 3-log reduction of viable cell numbers of wild-type (WT) strains and a 4-log reduction of cell counts pertaining to the MDR-TB strain $mc^2$5857. Based on these results, IVM has a bactericidal effect on some strains of *M. tuberculosis*. IVM also inhibited the proliferation of *Chlamydia trachomatis* on epithelial cells (HeLa 229) [39]. Production of infectious elementary bodies (EBs) and chlamydial 16 s rRNA was inhibited when exposed to 5 µM IVM [39]. This is important, as chlamydiae have a biphasic life cycle [40, 41], with metabolically inert EBs acting as infective agents that mature to metabolically active, but non-infectious, reticulate bodies giving rise to infectious EBs [42, 43]. An IVM concentration of 5 µM decreased the size of *C. trachomatis* inclusions, whilst 10 µM completely inhibited inclusion development [42]. Chlamydial maturation occurs in a host cell vacuole termed the chlamydial inclusion [42], and as such, inclusion suppression correlates with infection suppression.

In vitro studies have shown that the levels of IVM required to slow down the growth or kill *S. aureus*, *M. tuberculosis* and *C. trachomatis* (Table 1) are much higher than the recommended oral dosages effective in the treatment of parasites (0.046 µg/mL; 0.5 µM) [35] and SARS-CoV-2 (0.438 µg/mL; 5 µM) [33]. It is well documented that 93% of IVM administered orally binds to serum albumin, which leaves only 7% available to react with bacterial cells [43]. Clearly, this refutes efforts to use IVM as an orally administered antibacterial drug.

Table 1 Levels of IVM required to act antibacterially compared to predetermined concentrations of the drug required to act as an anthelmintic (0.046 µg/mL; 0.5 µM) and anti-SARS-CoV-2 agent (0.438 µg/mL; 5 µM)

MIC levels of IVM, as determined in vitro	Reference
S. aureus (6.25–12.5 µg/mL)	[36]
S. aureus (20.0 µg/mL)	[37]
M. tuberculosis (1.5–16.0 µg/mL)	[38]
C. trachomatis (0.44 µg/mL)	[39]

Possible Mode of Antibacterial Activity of IVM

IVM interacts with IMPα/β1 and by doing so prevents the translocation of viral particles into the nucleus. Prokaryotes do not have an IMPα/β1 transport system or a homologue of this system, which implies that IVM must have a different target in sensitive bacterial cells. Ivermectin (Fig. 1) is classified as an anthelmintic macrocyclic lactone (ML) [44] and is similar in structure to a class of drugs known as macrolides that have antibiotic properties. However, unlike macrolides, MLs contain no deoxy sugars attached to the macrolide ring backbone [45]. Research conducted by Koyama et al. [46] on the ML albocycline and its effect on MRSA may shed some light on the antibacterial mode of action of IVM. By using radiolabelled precursors of [^{3}H]thymidine, [^{3}H]uracil, [^{3}H]leucine and [^{3}H]GlcNAc, the authors discovered that albocycline prevents the incorporation of [^{3}H]GlcNAc into macromolecules. Based on these findings, the authors suggested that albocycline blocks peptidoglycan synthesis. IVM may act in a similar way. Scanning electron microscopy (SEM) images have shown structural changes of cell walls (wrinkles and sagging) when *S. aureus* cells were exposed to 4 × the MIC of IVM (80 µg/mL) [37]. This confirmed the hypothesis that IVM interferes with cell wall synthesis. Leaking of cellular contents was also visible in SEM images [37]. Transmission electron microscopy (TEM) has shown that *S. aureus* cells exposed to 80 µg/mL IVM form intracellular trachychromatic aggregates. This was confirmed by the leaking of uranyl acetate across damaged cell walls [37]. Staining of the DNA of damaged cells with propidium iodide confirmed changes in the permeability and integrity of cell walls [37]. These findings may also explain the bactericidal activity recorded with IVM against *M. tuberculosis* [38]. The peptidoglycan content of *M. tuberculosis* is similar to that of *S. aureus* [47]. Cells of *M. tuberculosis* are, however, protected by an acid-fast capsule, and IVM would have to penetrate, or damage, the outer layer. More research will have to be conducted to determine if other IVM target sites exist in bacteria.

Sensitivity of Gut Microbiota to IVM

The human gut hosts close to 4 trillion microorganisms and represents between 400 and 500 species [48, 49]. The composition of gut microbiota changes with age and is affected by diet, medication, hormonal changes

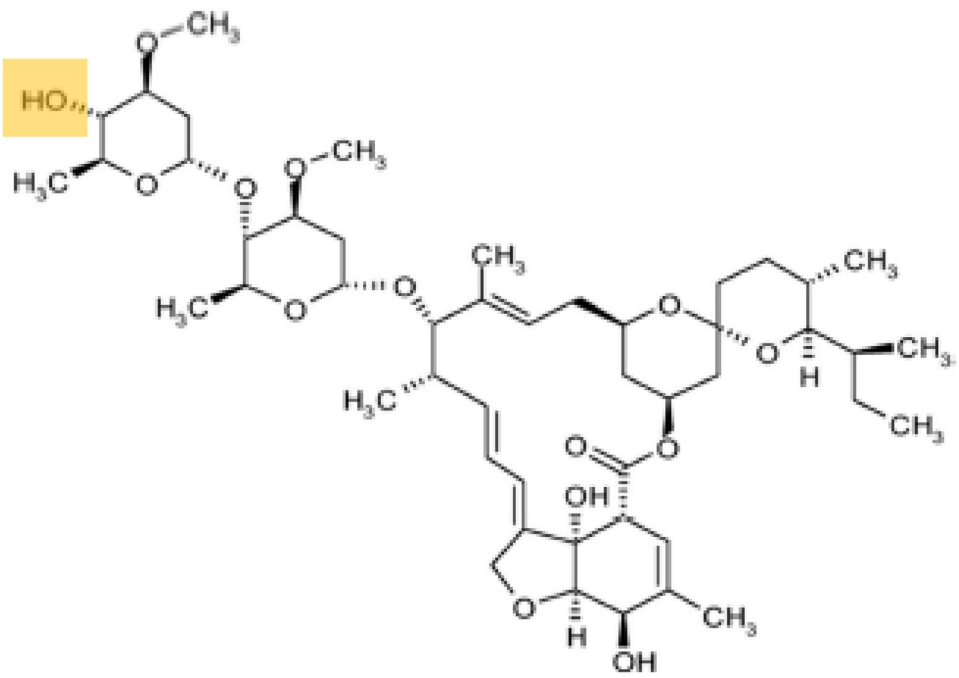

Fig. 1 Chemical structure of ivermectin (IVM). The hydroxyl group highlighted in yellow was changed to an amino (NH_2) group in the study conducted by Tan et al. [37]

and environmental stress [50]. Despite this, the adult gut has a common core of microbiota autochthonous to the gastrointestinal tract (GIT) [51], mostly consisting of genera belonging to *Firmicutes* and *Bacteroidetes* [52, 53]. Beneficial microbiota regulate gut wall permeability and modulate the immune system, but some have antibacterial and antiviral properties and keep the gut microbiota in a homeostatic state (reviewed by Dicks and Botes [54], van Zyl et al. [55] and Dicks and Grobbelaar [56]). Drastic changes in gut homeostasis may lead to inflammation caused by normal commensal microorganisms and pathogens. To the best of our knowledge, no in-depth studies have reported on the direct effect IVM has on gut microbiota and we do not know if continuous exposure to the drug could lead to dysbiosis. Several studies, reviewed by Dicks et al. [50], Chey and Menees [57] and Liu et al. [58], have shown that dysbiosis may lead to IBS (irritable bowel syndrome), enterocolitis and diarrhoea. An abnormal, or disturbed, gut microbiome may lead to the developing of neurological and psychiatric diseases, including anxiety, depression, major depressive disorder (MDD), schizophrenia, bipolar disorder, autism and obsessive–compulsive disorder (OCD) [59].

IVM is normally taken orally, which implies that prolonged dosage may lead to an imbalanced oral microbiome. Oral microorganisms play an important role in the developing of the gut microbiome, as shown in a recent study that linked first-phase schizophrenia, associated with gut dysbiosis, to changes in the salivary microbiome [60]. The study involved 208 individuals diagnosed with symptoms of first-phase schizophrenia and psychosis (high risk schizophrenia) and a group without psychiatric disorders. Individuals diagnosed with first-phase schizophrenia had a much higher number of *Firmicutes* compared to *Proteobacteria*, similar to what has been recorded in the salivary microbiome of patients with primary Sjögren's syndrome [61], an autoimmune disease involving chronic inflammation of the salivary and lacrimal glands. Qing et al. [60] suggested that these patients had higher cell numbers of microorganisms with the ability to produce branched-chain amino acids (BCAA) and lysine. This may explain the increase in cell numbers of *Staphylococcus* and *Megasphaera* in schizophrenic individuals. Species from both genera produce BCAA and lysine [62, 63]. Since strains of species present in the oral cavity have been isolated from the large intestine [63–65], changes in the oral microbiome inflicted by IVM may have a profound effect on gut and mental health. It should, however, also be noted that SARS-CoV-2 infection, treated or not treated with IVM, may in any case lead to the abnormal shedding of oral microbiota [66] and dysbiosis of the GIT.

Schneeberger et al. [67] studied the effect of anthelmintic drugs (both alone and in combination therapy treatment regimens) on the gut microbiome of adult individuals infected with hookworm. After 24 h of treatment with orally administered tribendimidine (400 mg), combined with IVM (200 µg/kg), cell numbers of *Bacteriodetes* increased in individuals that received only IVM. The treatment groups receiving tribendimidine plus IVM showed no signs of bacterial inhibition, as the entire bacterial abundance between all phyla displayed no significant change. The two families found to account for the largest variations within the *Bacteriodetes* phylum were *Prevotellaceae* and *Candidatus homothermaceae*. The increase in *Prevotellaceae* may have a detrimental effect on human health, since members of this phylum are known to be opportunistic pathogens [68] targeting and disrupting mucosal layers and destroying protective barriers. *Prevotellaceae* are also dominant in individuals diagnosed with IBD [68–71]. Studies conducted on mice showed that *Prevotellaceae* reduce short-chain fatty acid (SCFA) levels in the GIT, which in turn leads to a decrease in interleukin (IL)-18 production and an increase in intestinal inflammation [72]. Schneeberger et al. [67] also reported an increase in biotin metabolism and folate and N-glycan biosynthesis 24 h after treatment with IVM. These pathways are all involved in the synthesis of B vitamins. This finding may be explained as cell numbers of *Candidatus homothermaceae*, which regulates vitamin B synthesis in the GIT of mammals [73], increased 24 h after treatment with IVM combined with tribendimidine. This may be beneficial to the host, as B vitamins act as cofactors and coenzymes in multiple metabolic pathways and aid in keeping the immune system balanced [74]. Treatment with only tribendimidine did not produce the same results observed with a combination of tribendimidine and IVM, suggesting that changes were caused by IVM or possibly by a synergism between the two drugs. Three weeks after treatment, bacterial cell numbers and relative abundance returned to pre-treatment levels [67], suggesting that IVM has a limited effect on gut microbiota, the immune system and vitamin B production. No changes in the population of gut microbiota were observed when individuals were treated with tribendimidine (400 mg), tribendimidine (400 mg) plus oxantel pamoate (25 mg/kg) and albendazole (400 mg) plus oxantel pamoate (25 mg/kg).

A study conducted on Amur Tigers showed that treatment with fenbendazole (2 500 mg) plus ivermectin (100 mg) resulted in a significant increase in the relative abundance of *Firmicutes* and *Proteobacteria* post treatment, whilst *Actinobacteria* levels decreased drastically [75]. Cell numbers of *Collinsella*, *Clostridium XI* and *Megamonas* decreased, whilst cell numbers of *Escherichia* and *Clostridium* sensu stricto increased. These changes led to several biochemical alterations and thus altered the tigers' metabolic phenotypes. The concentration of five metabolites that were present before treatment increased significantly, whilst the concentration of 10 metabolites decreased. Although the authors did not elaborate on the benefits and disadvantages of these changes, treatment with a combination of fenbendazole and IVM was

considered advantageous, as cell numbers of pathogenic species from the *Clostridium* XI cluster were replaced by members of the *Clostridium* sensu stricto cluster, a cluster that has been documented for its gut-modulating abilities [76, 77]. Lowering of *Collinsella* numbers is considered beneficial, as they decrease the expression of tight junction proteins and may cause a leaky gut [78].

To the best of our knowledge, no information is available on the effect IVM has on beneficial gut microorganisms, especially probiotic lactic acid bacteria (LAB). A key factor in the survival and persistence of bacteria in the GIT is the ability to form biofilms [79]. Biofilm formation by LAB protects the GIT from bacterial and viral infections [55, 56] and keeps the gut wall impermeable [80]. Biofilm formation is beneficial to the proliferation of gut microorganisms [79] and human health [55, 56, 80]. In the study by Tan et al. [37], IVM at 40 µg/mL did not inhibit biofilm formation by the MRSA strain ATCC 43,300. However, treatment with IVM at 4 × MIC had a negative impact on the mRNA transcription of *relQ*, *rsbU*, *spA*, *icaD* and *sigB*, all genes associated with *S. aureus* biofilm formation. The genes were downregulated by 0.37–0.40, 0.23–0.28, 0.27–0.405, 0.0004–0.00155 and 0.60-fold, respectively. Similar results were reported for MRSA strain TCH1516 [81]. Although the authors have shown that 6.8 µM IVM inhibited the growth of *S. aureus* TCH1516 (IC_{50} value), 20 µM IVM did not prevent biofilm formation. What these studies show is that IVM, even at relatively high experimental concentrations, is unable to inhibit *S. aureus* biofilm formation. More research on biofilm formation must be done. The bacteriostatic activity of IVM against *S. aureus* [36] is a concern, as biofilm-forming strains may develop resistance [81, 82]. Apart from being protected from the host's immune responses, biofilms are more resistant to antibacterial drugs [82], which necessitate the search for more effective treatment of infections.

Conclusions

The use of IVM increased drastically since the outbreak of COVID-19 due to the publication of papers that suggest it may be used to combat SARS-CoV-2 infection. Conflicting reports on the efficacy of IVM inhibiting the proliferation of SARS-CoV-2 have been published. Despite scientific proof that dosage levels required for IVM to have a systemic effect on SARS-CoV-2 are well above that approved by the FDA, many believe that it has curing properties. The antibacterial properties of IVM, although evidence of this is based on in vitro tests, are of concern as prolonged use may lead to gut dysbiosis. If IVM does indeed affect peptidoglycan synthesis, in-depth studies need to be done to determine the effect prolonged use has on gut microbiota and the possibility of developing resistant strains.

Declarations

Conflict of Interest The authors declare no competing interests.

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