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REVIEW ARTICLE

Chemotherapeutic Potential of Monensin as an Anti-microbial Agent

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Abstract: Monensin is a lipid-soluble naturally occurring bioactive ionophore produced by *Streptomyces* spp. Its antimicrobial activity is mediated by its ability to exchange Na^+ and K^+ ions across the cell membrane thereby disrupting ionic gradients and altering cellular physiology. It is approved by Food and Drug Administration as a veterinary antibiotic to treat coccidiosis. Besides veterinary applications, monensin exhibits a broad spectrum activity against opportunistic pathogens of humans such as bacteria, virus, fungi and parasites in both drug sensitive and resistant strains. This ionophore can selectively kill pathogens with negligible toxic effect on mammalian cells. In this review, we discuss the therapeutic potential of monensin as a new broad-spectrum anti-microbial agent that warrants further studies for clinical use.

Keywords: Monensin, Anti-microbial, Ionophores, Pathogens, Chemotherapy, Drug-resistant.

1. INTRODUCTION

Ionophores (ion carriers) such as 'monensin' are small lipophilic and hydrophobic molecules, which form complex with specific inorganic ions or biogenic amines and can increase their permeability across biological and artificial lipid vesicles. These ionophores have played a significant role in understanding ion transport mechanism across the membrane, a major biochemical phenomenon that operates in higher to lower biological organisms [1]. Ions are transported across the cell membranes through ion pump or channel proteins which helps to maintain ionic gradient for cellular function. The ionophores disrupt ionic gradient leading to alteration in function depending on the specific gradient. The purpose of the review is to understand the applications of monensin as a chemotherapeutic agent against various pathogenic organisms. Ionophores are classified as neutral ionophores (*e.g.* valinomycin), carboxylic ionophores (*e.g.* monensin) and quasi-ionophores (*e.g.* gramicidin). Naturally occurring polyether antibiotic, Monensic acid was discovered for its profound anticoccidial activity [2], isolated from its natural source *Streptomyces cinnamonensis* [3].

Monensin are related to the crown ethers which form complexes with monovalent cations such as Li^+, Na^+, K^+, Rb^+, and Ag^+ [4]. The most notable feature of monensin is capable of forming complexes with cations and transport across phospholipid cell membranes as Na^+/H^+ antiporter.

2. STRUCTURE

Monensin is an open chain molecule with three tetrahydrofuran and one tetrahydropyran ring with group at one terminal and tertiary hydroxyl group at the other end (Fig. **1**). The molecule forms complex with ions through its cation ligand binding sites composed of oxygen atoms of the tetrahydrofuran and tetrahydropyran rings. Since the ionic radius is determinative in net free energy difference between desolvation and complexation, the ionic radius of sodium ion (0.95Å) permits it to fit exactly into this cavity. These results have ten times higher affinity of monensin for sodium ions than potassium ions (ionic radius 1.33 Å). Cationic complexation results in central orientation of the ligand bound sites with nonpolar hydrocarbon backbone on the surface of the complex. This structural arrangement minimises interaction of the bound cation with solvent thereby rendering the complex soluble only in nonpolar solvents. In addition, this solubility characteristic is responsible for the ability of monensin to transport sodium ion across lipid bilayer (Fig. **1**). The nonpolar complex is soluble in the acyl residues of the lipid bilayer, thus allowing it to diffuse across aqueous membrane interface [5].

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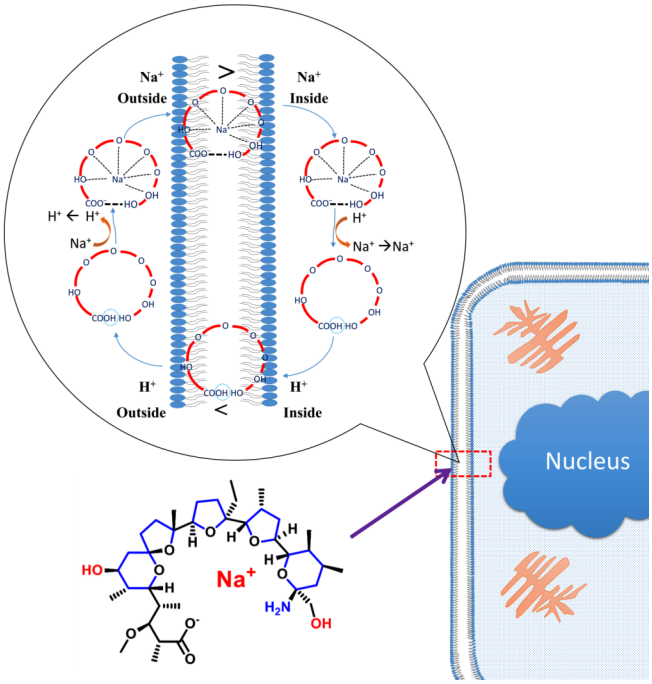

Fig. (1). Structure of monensin A and their transport mechanism across the cell membrane. Monensin acts as antiporter by mediating the exchange of Na^+/H^+ ions and affects the cytosolic pH.

3. BROAD SPECTRUM ACTIVITY OF MONENSIN

Monensin has immense therapeutic potential as anti-bacterial, antifungal, anti-parasitic, antiplasmodial, anti-viral, anti-trypanosomiasis, anti-toxoplasmosis and anti-leishmaniasis agent due to its ability to disrupt physiological functions in organisms. In the forthcoming sections, we will elaborate on the clinical importance of monensin against various diseases.

4. MONENSIN AS AN ANTI-BACTERIAL AGENT

The antimicrobial action of monensin is dependent on the cell membrane composition of the microbes. Monensin affects Na^+/H^+gradient across the membrane and affects the osmotic gradient of the cell leading to irreversible growth arrest [6]. The native form of monensin was found to be effective in inhibiting the growth of diverse pathogenic gram (+ve) bacterial strains. To improve its efficacy,the backbone of monensin has been modified with addition or removal of functional groups that lead to enhanced antimicrobial effect as well reduced toxic-effects. The ester derivatives of monensin containing morpholine ring moiety exert antibacterial activity against both methicillin resistant and susceptible strains of *Staphylococcus aureus* as well as other strains of human pathogenic bacteria. Monensin derivatives with phenyl or naphthalene ring showed decreased effectiveness against the gram-positive bacteria. New derivatives of monensin synthesized using double modified ester-carbonate were able to inhibit the growth of *S. aureus* strains (MIC: 4.8-21.6µM) and *Staphylococcus epidermidis* (MIC: 9.7-21.6µM). Similarly double modified amide-carbonate derivative blocked the growth of *S. aureus* strains (MIC: 81.4-162.8µM) and *S. epidermidis* (MIC: 325.7µM). But these double modifications increased the concentration of antibiotic needed to inhibit the growth of *Staphylococcus* strains in comparison to the unmodified monensin [7]

Regio-selective chemical modification alters the ionic selectivity of monensin with differential killing effect on bacteria. An amide derivative, monensin N–phenylamide forms specific complex with Na^+ ions. It was comparably effective against clinical bacterial isolates as that of monensin ester derivative [8]. On the contrary, amide derivative was not effective in inhibiting the growth of *Enterococcus hirae* even at a concentration higher than 400µg/ml. Apparently, gram-negative bacteria were insensitive to the amide derivative similar to monensin A due to their higher molecular weight and hydrophobic nature [9]. Monensin N-phenylamide was potentially active against human opportunistic pathogenic strains, *S. aureus* and *S. epidermidis* [10].

The monovalent complex of monensin N-allylamide showed profound antibacterial activity against *Staphylococcus* sp. and *Bacillus* sp. than the allyl derivative of monensin [8, 11]. But they were less efficient relative to phenylamide derivative of monensin. In addition, urethane derivatives of monensin display potent antibacterial activity than unmodified monensin and are even able to transport the divalent ions across the membrane [12]. Addition of urethane substituent to monensin sodium markedly improved the biological activity against hospital strains of methicillin-resistant *S. epidermidis* and *S. aureus* in comparison to unmodified parent compound [13]. Similarly, monensin reduced the viability of methicillin-resistant *S. aureus* biofilm in treating bovine mastitis with minimum inhibitory concentration (MIC90) values less than16 µg/ml [14]. Monensin substituted with 4-aminobenzo-15-crown-5 improved the metal complex forming ability because of their two hydrophilic sites. The substituted monensin transport Na^+ in the ratio of 1:2 but with a lower biological efficiency than monensin A. This phenomenon is due to increased size and electrogenic potential which in turn reduce their biological activity against gram-positive bacteria [15]. Concordantly, Monensin hands its derivative containing thallium (I) showed equal efficacy against tuberculosis causing pathogen, *Mycobacterium tuberculosis* H37Rv with 97-99% growth inhibition at a concentration of 6.25µg/ml [16]. Similarly, other metal complexes of monensin showed profound inhibitory effect against *M. tuberculosis* H37Rv. The monensin complex with metals such as Ba^{2+} $Mg^{2+,}$ Ca^{2+}, Sr^{2+}, Cs^+, Li^+ exhibited enhanced killing activity with MIC values in the range of 0.23-0.73 µg/ml against *M. tuberculosis* H37Rv. It showed dose-dependent inhibition of *M. tuberculosis* complex in culture with greater potency relative to BCG. Also, the study demonstrates that monensin is an effective inhibitor of *Mycobacterium avium* subspecies paratuberculosis (MAP) in radiometric culture with MIC of 0.39µg/ml [17]. The anti-MAP activity of monensin could be due to perturbation in the metabolic pathways of *M. tuberculosis* with no effect on cell wall. Furthermore, treatment with monensin sodium effectively reduced the growth of bovine paratuberculosis with minimal pathological symptoms in murine model system [18]. Other complexes of monensin with metals such as Rb^+, K^+, Na^+, Cu^{2+} possess anti-tubercular activity with comparatively higher MICvalues greater than 1 µg/ml. The enhanced activity of the monensin metal complexes is due to their improved hydrophobicity

and increased permeability across membrane [19] causing detrimental effect on bacterial growth.

Monensin inhibited the growth of other intracellular pathogens such as *Klebsiella pneumonia* and Brucella through inhibition of endosome acidification [20]. Likewise, monensin disrupted the intracellular growth of *Legionella pneumophila* at a concentration of 25 µM by affecting the intracellular pH of the vacuole and thereby altering the iron metabolism [21]. Thus, monensin facilitates the killing of numerous intracellular pathogenic bacteria that evaded the immune system as shown in Fig. (**2**).

The additive effect of monensin with synthetic enterocin (bacteriocin) CRL35 was proven against pathogenic bacteria, *Listeria monocytogenes* FBUNT. The combined effect resulted in4-fold (MIC- 0.25 µg/ml) enhancement in their antibacterial activity than monensin alone (MIC- 1µg/ml) [22]. These results substantiate the efficacy of monensin based combination therapy in the treatment of foodborne pathogen and prevent the selection of drug resistance among the pathogens.

The potential role of monensin to bind with the divalent cations such as Co^{2+}, Mn^{2+} [23], Ca^{2+}, Mg^{2+}, $Ni^{2+}$and Zn^{2+}has been well studied.However, the transition and alkaline earth metal complexes of monensin showed an improved activity against *B.subtillis, B. mycoides* and *S.lutea*. The presence of calcium, magnesium, nickel and zinc in complex with parent monensin demonstrated 17-fold enhanced activity than unmodified monensin ligand with the MIC_{50} value of 0.7-1.4 µM [24]. Furthermore, manganese and cobalt complexes of monensin showed 2-fold elevated activity against gram (+ve) bacteria [23] than the uncomplexed form of monensin. These complexes enhanced the inhibitory effect on the growth of gram-positive bacteria [25]. Notably, increasing concentration of metal ions (Na^{+}) in growth medium potentiated the efficacy of monensin against selected species of ruminal bacteria. Thus, dietary supplementation of monensin with ions could enhance the inhibitory effect against ruminal organisms in treating cattle [26]. The C-26 modified monensin (26-phenylaminomonensin) and 7-O-(4-substituted benzyl)monensin showed stronger antibacterial activity against diverse bacterial pathogens than the parent compound [27].

Though monensin is ineffective against most of the gram-negative bacteria, it has displayed antibacterial activity against anaerobic gram-negative flora, *Bacteroidesfragilis* ATCC 23745 (MIC: 1.56 µg/ml). Also, anaerobic gram-positive strains including *Clostridium* sp., *Eubacterium* sp., *Peptococcus* sp. and *Peptostreptococcus* sp., were sensitive to the ionophore monensin [28]. Interestingly, various clinical isolates of gram-positive bacteria are highly susceptible to monensin and a few other urethane derivatives of monensin (Table **1**). Though monensin and its derivatives show promising antibacterial activity against the gram-positive bacterial isolates, neither monensin nor its deriva-

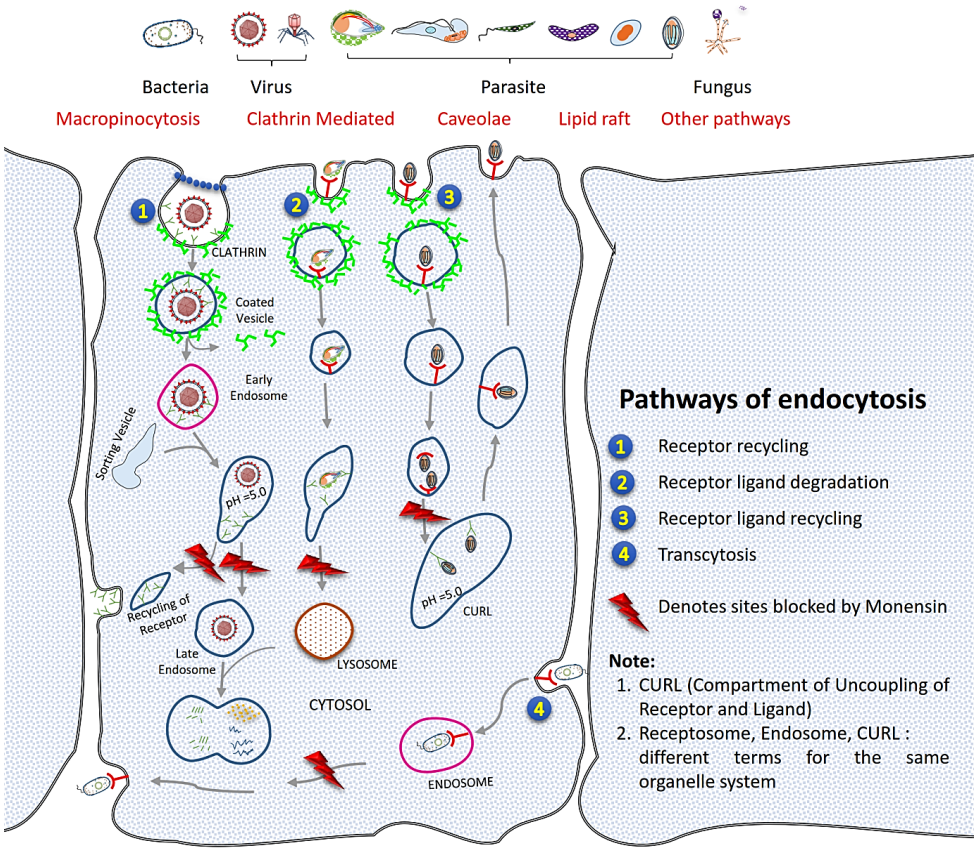

Fig. (2). Entry mechanism of different types of intracellular obligate pathogens through a variety of endocytic processes. Monensin exerts inhibitory effect on diverse pathogens by affecting the pH of endosome-lysosome system. Treatment with monensin causes irreversible growth arrest by blocking their invasiveness and multiplication.

Table 1. The effect of monensin and its derivatives on various bacterial pathogens.

Monensin Derivative	Bacterial Strains	MIC (µg/ml)	References
Monensin ester with morpholine group	*Staphylococcus aureus, Staphylococcus epidermis*, *Bacillus subtilis, Bacillus cereus, Enterococcus hirae, Micrococcus luteus*	6.25-50	[8]
Monensin ester with allyl group	*S. aureus, S. epidermis*, *B. subtilis, B. cereus, M. luteus*	12.5-100	[8]
Monensin N–phenylamide	*S. aureus, S. epidermis*, *B. subtilis, B. cereus, M. luteus*	6.25-12.5	[9, 10]
Monensin N-allylamide	*S. aureus, S. epidermis*, *B. subtilis, B. cereus, M. luteus*	25-100	[11]
Monensin amide (4-aminobenzo-15-crown-5)	*S. aureus, S. epidermis*, *B. subtilis, B. cereus, M. luteus*	25-50	[15]
Monensin urethane-Phenyl	*S. aureus, M. luteus, Streptococcus faecium, Bacillus sp. E, B. subtilis, B. megaterium, Mycobacterium pheli, Streptomyces celluolosae, Paecilomycesvarioti*	0.1-3.1	[12]
Monensin urethane-methylphenyl		0.05-3.1	
Monensin urethane-Fluorophenyl, Bromophenyl		0.05-1.6	
Monensin urethane-Iodophenyl		0.02-1.6	
Monensin urethane-Chlorophenyl		0.02-3.1	
Monensin urethane-Nitrophenyl		0.1-1.6	
Monensin urethane- Cyclohexyl, Phenoxyphenyl		0.1-3.1	
Monensin urethane-(R) and (S)-1-phenethyl		0.1-12.5	
Monensin urethane- Methyl		1.6 - >25	
Monensin urethane- 2-phenethyl		0.4 - >25	
Monensin A phenylurethane sodium salt	methicillin-resistant *S. epidermidis* and *S. aureus*	0.5-1	[13]
Monensin dipodand	*S. aureus, S. epidermis*,*B. subtilis, B. cereus, M. luteus, E. hirae*	6.25-100	[29]
Monsenin tripodand		12.5-200	

tives were effective against the gram-negative bacteria. This major setback is due to the structural integrity of bacterial outer membrane which inhibits the entry of high molecular weight, hydrophobic monensin across cell bilayer. Further studies are needed to identify monensin derivatives which could be effective against the gram-negative bacteria.

5. MONENSIN AS AN ANTIFUNGAL AGENT

Monensin exhibits dual activity against the rumen fungus *Neocallimastix sp.* LM1. At lower concentration (1µg/ml), it acts as fungistatic and at higher concentration (16µg/ml) as fungicidal [30]. Monensin interfered with gametes and zoospore formation in aquatic fungus *Allomycesmacrogynus*by disrupting the function of Golgi cisternae which plays critical role in gametogenesis and zoosporogenesis [31]. The growth of fungi (*Botrytis cinerea* and *Sclerotiumrolfsii*) was inhibited by monensin through perturbation of the exopolysaccharide secretion and vesicular trafficking [32]. Monensin impaired the growth of yeast by disrupting the functions of intracellular transport, late secretory pathways and acidic vacuole. It also inhibited the cell wall synthesis of protoplast forming *Neurosporacrassa* [33]. In addition, monensin interrupted the growth of various fungi (*Hypomyceschlorinus*, *Neurosporacrassa*, *Achlyabisexualis*, and *Taphrinadeformans*) by inhibiting the sterol biosynthesis [34] . Notably, monensin perturbed the growth of human pathogen *Candida albicans* by disrupting the vacuolar function leading to cell death [35]. Monensin A ester derivative inhibited the growth of Candida [8]. Moreover, effect of monensin was tested on yeast knockout mutants involved in post-Golgi traffic indicating the interference in late secretory pathway by altered pH [36]. Thus, there are differences in the mode of action of monensin to inhibit the growth of different fungal strains.

6. MONENSIN AS AN ANTIPARASITIC AGENT

Monensin restrains the growth of clinically important human parasites such as *Plasmodium falciparum, Trypanosomacruzi*, *Toxoplasma gondii*, *Leishmaniadonovani and Dirofilariaimmitis* as shown in Fig. (**2**). Monensin is widely used in poultry industry to control parasites especially to prevent coccidial infections demonstrating its efficacy against both single and mixed species infection of *Eimeria* [37]. Besides, its use in poultry, it controls coccidiosis in game birds, sheep and cattles [38].

7. MONENSIN AS AN ANTIPLASMODIAL AGENT

Several studies have demonstrated that monensin inhibited blood stage forms of human malaria parasite (*Plasmodium falciparum*) both *in vitro* and *in vivo* conditions by alkalization of its digestive vacuole. [39] Being lipophilic in nature, monensin intercalates into lipid bilayers and mediates influx of monovalent cations (K^+or Na^+) with efflux of H^+ thereby altering the pH of food vacuole. It completely inhibits its food vacuole protein degradation by increasing intravesicular pH of parasite [40]. Interestingly, normal erythrocytes have low level of Na^+, high level of K^+ and low level of Ca^{2+}in the cytosol. In contrast, cytosol of malaria-infected erythrocytes contains high level of Na^+, low level of K^+. Parasite cytosol contains high level of K^+ and low level of Na^+ with 20-40 fold higher Ca^{2+} contents than the infected erythrocytes [41]. Thus accumulation of sodium or potassium by monensin impaired the growth of parasite by reducing the digestion of hemoglobin. The change in ionic equilibrium affects parasite cellular functions by distorting cytoskeleton, causing imbalance in levels of intracellular messengers like Ca^{2+} and cAMP and cause irreversible growth arrest by inhibiting mitotic division. There is a direct relationship between antiplasmodial activity and the ion flux induced by monensin in infected human erythrocytes. Also, presence of C26-hydroxyl group of the molecule is essential for the stability of 1:1 sodium complex of natural monensin and for its intrinsic antimalarial activity [42].

Among various potentantimalarial drugs reported to date, monensin exhibited remarkable activity *in vitro* against blood stages of various clinical isolates of *P. falciparum* with lower IC_{50} values in the range of (1.1 - 2.3nM) [43]. A semisynthetic urethane derivatives of monensin showed marked inhibitionof *P. berghei*infection and improved thesurvival in mice [44]. Interestingly, monensin exhibited selectivecytotoxic effects on *Plasmodium* infected erythrocytes while sparing normal erythrocytes [45]. In other study, monensin displayed selective killing ability of infected erythrocytes [46]. Compounds identified through quantitative structure-based activity relationship (QSAR) model showed that monensin had superior activity among compounds tested such ashycanthone, amsacrine, aphidicolin, bepridil, amiodarone, ranolazine and triclocarban against blood stages of *P. falciparum* 3D7 clone [47]. Besides, monensin effectively cleared the blood parasite load in mice infected with *P. chabaudi and P. vinckeipetteri*, an ideal rodent model for studying the liver stage malaria. Interestingly, monensin showed several fold enhancements in antiplasmodial activity compared to standard antimalarial drugs (chloroquine, artemisinin) in *in vitro*. Monensin in combination with artemisinin exhibited synergistic to additive effect on growth of cultured *P. falciparum* [48]. Additionally, combined treatment of monensin and nigericin resulted in synergistic effect both *in vitro* and *in vivo*. Monensin exchanges Na^+ for protons while nigericin exchanges K^+ for protons leading to enhanced acidification of parasite cytosol [48b]. Also, combination therapy of monensin with other antimalarial drugs (chloroquine, piperaquine, FR900098) effectively inhibited parasite growth acting either synergistically or additively in cultured *P. falciparum* strains and *P. berghei* infection in mice [49].

As an antimalarial agent, monensin targets the parasite at multiple life stages. There are also evidences suggesting that pre-treatment of mice or HepG2 cells with monensin leads to impairment of sporozoite invasion [50]. Pre-treatment with monensin induced remodelling of the host cell (hepatocyte) resulting in protection against liver stage of malaria (exo-erythrocytic stage). Also, monensin could obstruct liver stage infection of *Plasmodium berghei* at low nanomolar range potency [51]. Likewise, monensin treatment affected sporozoite attachment and motility from freshly isolated salivary glands of mosquitoes [52]. Surprisingly, monensin was able to completely inhibit *P. vivax* hypnozoites and schizonts suggesting its effectiveness in treating liver stage malaria [53]. Also, monensin could block the transmission of gametocytes at the sexual stage [54]. Malarial parasites treated with monensin led to accumulation of transport vesicles and blockade of vacuole-vesicle fusion suggesting that monensin targeted endocytic pathway [55]. In addition to these effects, monensin induced 'Eryptosis'in plasmodium infected erythrocytes leading to cell shrinkage and scrambling of cell membrane demonstrating a new mechanism of cell death [56]. Therefore, the therapeutic efficacy of monensin as an anti-malarial agent is mediated by depletion of proton levels inside acidic food vacuole and disruption of vesicular trafficking in parasites. Chemically modified monensin (phenylurethane and chlorophenylurethane) inhibited the growth of *P. berghei*in mice [12]. Thus, lysosomotropic alkalinizing agent- monensin acts as a strong antiplasmodial agent without affecting the host cells at therapeutic dosage.

8. MONENSIN AS AN ANTI-TRYPANOSOMIASIS AGENT

Insect transmitted protozoan parasite *Trypanosoma* leads to sleeping sickness leading to high mortality. Monensin interfered with the growth of *Trypanosoma cruzi* (epimastigote and trypomastigote) by affecting parasite-macrophage interaction due to alteration in membrane components [57]. Monensin increased the intravesicular pH of parasite entered mammalian cells thereby inhibiting the exit of *T. cruzi* from phagosome [58]. Interestingly, monensin impeded the growth of *T. brucei*,by blocking the synthesis and transport of variant surface glycoprotein (VSG) [59]. Monensin and its derivatives inhibited the bloodstream forms of *T. brucei* with lesser toxicity in human HL-60 cells and higher selectivity index [60]. Moreover, monensin inhibited starvation-induced autophagic activity and acidocalcisomes in *T. brucei* [61]. Thus, monensin hampers the growth of different species of *Trypanosoma* by different functions.

9. MONENSIN AS AN ANTI-TOXOPLASMOSIS AGENT

Monensin was found to be effective on both sexual and asexual stages of *Toxoplasma*. Exposure to monensin altered cellular physiology and leads to autophagy-like cell death in *Toxoplasma gondii* culture [62]. The parasiticidal effect of monensin was observed against the cyst form (bradyzoite) of *T. gondii*. Monensin impaired the growth by forming swollen vesicles which led to cell lysis [63, 64]. Notably, TgMSH-1-dependent cell cycle disruption during the late-S-phase by

monensin is a new mechanism of cell death in *T. gondii* [65]. However, loss of TgMSH1 (mitochondrial MutS DNA repair enzyme) in *T. gondii* failed to cause monensin induced cell death. This demonstrates that monensin kills parasites by inducing mitochondrial stress indicating a novel mechanism of action. Besides regulating cell cycle, monensin impaired mitochondrial function and induced oxidative stress in *T. gondii* [66]. More interestingly, monensin induced resistance in *T. gondii* tachyzoites resulted in reduced invasion and egress activities while increased intracellular replication. Their findings demonstrate upregulation of actin and down-regulation of microneme proteins (MIC8) are involved in attaining resistant phenotype leading to reduced growth rate [67].

10. MONENSIN AS AN ANTI-LEISHMANIASIS AGENT

Monensin exhibits a different mode of actions against growth stages of *Leishmania*. As a proton ionophore, monensin inhibited the entry of weak base into acidic compartments of megasomes and inclusion vesicles in amastigotes of *Leishmania amazonensis* [68]. The presence of monensin increased the pH of acidic vacuoles making it unfavourable for parasite survival [69]. *In vitro* studies showed that addition of monensin blocked the secretory acid phosphatise in *L.donovani* promastigotes demonstrating the inactiveness of the heterodispersenative form of enzyme (acid phosphatase) involved in golgimediated post-translational modification [70]. Also, monensin blocked the entry of promastigotes in murine macrophages by inhibiting ceramide concentration in *L.donovani* infected cells [71].

11. MONENSIN AS AN ANTI-CRYPTOSPORIDIOSIS AGENT

Monensin has been documented to block the growth of *Cryptosporodium,* one of the common water-borne parasitic diseases. Monensin completely inhibited the growth of *Cryptosporidium parvum* without causing toxic effects on mouse fibroblast cells (L929) [72]. In addition, monensin showed maximum growth inhibition of *C. parvum* by blocking the DNA replication in comparison to other anti-cryptosporidial drugs [73].

12. MONENSIN AS AN ANTI-COCCIDIOSIS AGENT

For several years monensin has been used as an anticoccidial agent and is marketed under the brand name of Elancoban. It exhibits anticoccidial activity by increasing the Na^+ ions inside acidic vacuole and disrupting the invasion event of sporozoites. Monensin bound to cation complex is highly effective against sporozoite and merozoite stage with rapid action against the coccidium *Eimeriatenella*. Monensin showed activity against the field isolates of *Eimeria spp*. in broiler farms and caused formation of distorted structures in *E. tenella* sporozoites(WIS strain) [74]In monensin susceptible strain of *E. tenella*, addition of monensin showed swollen and bulgy structures with loss of membrane fluidity in comparison to laboratory developed monensin resistant *E. tenella* [75]. Free merozoites of *E. Tenella* were rapidly killed by monensin due to osmotic swelling and cell burst while well differentiated schizonts and gamonts showed moderate growth inhibition [76]. Monensin fed chickens and turkeys were completely devoid of *E.mitis* and *E.dispersa* infections [77].

13. ANTI-PARASITIC EFFECT OF MONENSIN AGAINST OTHER PARASITES

Pre-treatment with monensin blocked TNFα secretion and activated the innate defence mechanism in mouse macrophage cell line (RAW 264.7) infected with DNA of intestinal protozoan *Entamoeba histolytica* [78]. In addition, monensin showed an inhibitory effect against a nematode infection, by affecting the contractile activity of *Dirofilaria-aimmitis*in dog heartworm [79]. Monensin exhibited activity against *Fasciola hepatica*-infected rats and adult tegument in culture [80]. The inhibitory effect was due to the perturbation of Golgi secretory pathway in the vitelline cells of *F. hepatica* [81]. These studies clearly demonstrate the potential use of monensin in treating a variety of parasitic infections of clinical relevance.

14. MONENSIN AS ANTIVIRAL AGENT

Monensin exhibits antiviral activity against both veterinary and human disease-causing viruses. The most common viral infection in poultry birds caused by Newcastle disease virus (NDV) and Angara disease virus (ADV) could be treated with monensin. It showed immuno-modulatory effect by augmenting anti-NDV and anti-ADV responses suggesting its immune-potentiating property [82]. In addition, monensin blocks the cleavage of fusion protein (F_0) of Newcastle disease virus in the trans-Golgi membrane of the cell necessary for the virus propagation. Monensin prevented the low pH-dependent infection of *Equine arteritis* virus by interfering with the structural modification of membrane complex proteins of virus [83]. In addition, monensin hindered betanodavirus-induced cytopathology and virus production via inhibition of endosomal acidification [84]. It arrested the release of pseudorabies virus from the cell [85] by blocking the addition of fucose on the glycoprotein without disturbing the earlier glycosylation process.

The simian virus 40 belonging to polyoma family is known to cause different types of sarcomas. Monensin was effective in hindering endosomes mediated infection pathway in SV40 [86]. It prevented the acidification of the late endosomes necessary for further transport to endoplasmic reticulum. In addition, monensin has been reported to block the early stage of viral infection by preventing the internalization of virus [87]. Treatment of monensin inhibited the growth of Human Cytomegalovirus infection (HCMV) replication by decreasing the expression levels of IE2, UL44, and pp65 proteins [88]. Also, monensin prevented the generation of HCMV progeny by inhibiting DNA replication [89]. Marsh and co-workers [90] showed that monensin induced inhibition of Semliki virus penetration in BHK-21cells by increasing the pH (>6) in endocytic vacuoles and lysosomes. Similarly, monensin prevented the entry of Hepatitis C virus by inhibiting the fusion of viral and host cellular membranes [91].

Monensin showed potent anti-viral activity against the clinically important viral pathogen Japanese encephalitis virus (JEP) [92] as well as in combination with brefeldin A.

It inhibited the transport of virus from Golgi apparatus to the cell surface leading to the cleavage of precursor form of structural (M) protein. Furthermore, it obstructed the conformational changes in structural protein (E) which is essential during the infection process. Monensin inhibited the cleavage of Friend Murine Leukemia virus precursor envelope glycoprotein leading to defective endo-H-resistant oligosaccharides [93]. This caused a subsequent reduction in the virus yield as the virions lacked glycoproteins, gp70 and p15 (E) suggesting glycoprotein blockage at an early stage of viral growth. In another study, monensin treatment resulted in the production of non-infective Mason-Pfizer monkey virus particles due to the inhibition of cleavage of precursor glycoprotein ($Pr86^{env}$) [94] which is crucial for infecting new host cells. Monensin potentially hindered the cleavage of Hendra virus fusion (F) protein in trans Golgi network [95].

Vesicular stomatitis and Sindbis virus assembly and release were blocked by monensin as a result of complete inhibition of cell surface expression of viral envelope glycoproteins necessary for budding [96]. Monensin treatment delayed the transport of influenza virus surface proteins such as haemagglutinin and neuraminidase [97] across trans Golgi membrane to the plasma membrane [98]. Further, it affected the mannose processing of haemagglutinin and delayed the development of endo H-resistance. In contrary to influenza, the transport of glycoprotein was completely blocked in vesicular stomatitis virus by monensin [99]. It also blocked the transport and glycosylation of envelope proteins (E1 & E2) in coronavirus [100] thereby impairing the exocytosis of the virus from the cell.

Monensin demonstrated anti-HIV activity in MOLT-3 cells chronically infected with HTLV-IHB [101]. It inhibited the proteolytic cleavage of the env-coded polyprotein gpl60 to gpl20, leading to the accumulation of the precursor gpl60.The syncytia formed CEM cells (T Lymphoblast)co-cultivated with HIV-1-infected MOLT-3 cells markedly inhibited the viral growth when treated with monensin [102]. Monensinblocked the replication of HSV-1 [103] and human cytomegalovirus [104] *in vitro*. It blocked later stages of post-translational processing of glycoproteins in HSV-1 and HSV-2 [105]. It decreased the yield of both cell-associated and released Punta Toro virus particles in a concentration-dependent manner, which generally assembles at the Golgi complex restructure [106]. Besides regulating the protein modification and transport, monensin destabilized the early mRNA of mouse polyomavirus and inhibited the replication of the viral DNA [107]. In addition, monensin profoundly inhibited the vesicular stomatitis poliovirus protein synthesis without affecting the host cells (HeLa) translation machinery [108]. Altogether, monensin demonstrated strong antiviral activity either by blocking the virus entry or by preventing their release (Fig. **2**).

15. MONENSIN IN VETERINARY APPLICATIONS

In veterinary medicine, monensin was initially used to decrease methane production in the rumen of animals as methane. This ability of monensin is attributed to its potential to decrease the load of hydrogen-producing bacteria that required for methane production Thus feeding monensin to dairy animals reduced the production of methane [109]. It has been reported that presence of monensin in feeds of dairy animals caused inhibition of protein degradation to ammonia in cattle. Feeding monensin to cattle resulted in decreased feed consumption, high feed efficiency and improvement in daily gains [110]. This improvement in feed utilisation was due to increased efficiency of energy metabolism. Monensin induced change in the proportion of volatile fatty acid produced during microbial digestion in rumen has been suggested as the mechanism behind increased feed efficiency. There was an increase in propionic acid and concomitant decrease in acetic and butyric acid level in the rumen on feeding monensin [111].

Monensin has been approved by Food and Drug Administration (FDA), USA for use in cattle to increase feed utilisation under the trade name Rumensin. It was been reported that addition of monensin to diet significantly increased milk production in dairy cows [112] and decreased the percentage of milk fat and saturated fatty acid in Holsteins cows. It inhibits hypoglycaemia and ketonuria in cows [113]. By selectively eliminating lactic acid producing bacteria, monensin was able to control to acidosis effectively and decrease bloat formation in cattle [114]. Thus, monensin finds widespread application in the dairy industry. The action of monensin is just not restricted as the feed supplement but its potential as a therapeutic agent has also been exploited for the treatment of animal infections like coccidiosis, acidosis and bloat [110].

CONCLUSION AND PERSPECTIVE

Monensin offers diverse chemotherapeutic potential against various infectious agents. Repurposing monensin as anti-infective agent for humans may effectively ameliorate the outbreak of emerging pathogens for which there are no vaccines and drugs. Delivery of monensin using suitable nanocarriers may further enhance the therapeutic efficacy and minimize its non-specific toxicity. [115] This US-FDA approved veterinary antibiotic can be co-administered with standard chemotherapy in combating drug-resistant pathogens.This drug needs further pre-clinical evaluation [116], for treating human pathogens. Interestingly, another carboxylic polyether potassium ionophores salinomycin has been used in clinical trials in treating cancer patients.It showed partial regression of tumours with no observable severe side effects [117]. Therefore, further preclinical studies are warranted to determine the therapeutic potential of monensin for clinical use.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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