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Polyether ionophores—promising bioactive molecules for cancer therapy

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ABSTRACT

The natural polyether ionophore antibiotics might be important chemotherapeutic agents for the treatment of cancer. In this article, the pharmacology and anticancer activity of the polyether ionophores undergoing pre-clinical evaluation are reviewed. Most of polyether ionophores have shown potent activity against the proliferation of various cancer cells, including those that display multidrug resistance (MDR) and cancer stem cells (CSC). The mechanism underlying the anticancer activity of ionophore agents can be related to their ability to form complexes with metal cations and transport them across cellular and subcellular membranes. Increasing evidence shows that the anticancer activity of polyether ionophores may be a consequence of the induction of apoptosis leading to apoptotic cell death, arresting cell cycle progression, induction of the cell oxidative stress, loss of mitochondrial membrane potential, reversion of MDR, synergistic anticancer effect with other anticancer drugs, etc. Continued investigation of the mechanisms of action and development of new polyether ionophores and their derivatives may provide more effective therapeutic drugs for cancer treatments.

According to the World Health Organization (WHO) cancer is a leading cause of death worldwide, accounting for 7.6 million deaths (around 13% of all deaths) in 2008. Projections are even more disturbing because deaths from cancer are expected to rise to over 13.1 million in 2030.[1]

Over the last few decades, natural products have played a very important role as established cancer chemotherapeutic agents, either in their unmodified (naturally occurring) or synthetically modified forms.[2] Microorganisms are a prolific source of structurally diverse bioactive metabolites and have yielded some of the most important products in the pharmaceutical industry. These include antibacterial agents such as the penicillins, cephalosporins, aminoglycosides, polyether ionophores, tetracyclines, and other polyketides of many structural types of immunosuppressive agents, such as the cyclosporins and rapamycin. It is also well-known that antitumour antibiotics such as daunorubicin, doxorubicin, actinomycin, plicamycin, mitomycin, bleomycin, neocarzinostatin and lidamycin, etc. are the most important among the cancer chemotherapeutic agents.[3]

In 2009 Gupta et al. discovered that salinomycin, one of several used veterinary antibiotics that belongs to the large class of naturally occurring polyether ionophore antibiotics, is able to kill cancer stem cells, inhibit breast cancer growth and metastasis in mice.[4] Cancer stem-like cells (CSCs) in different types of cancers may account for the failure of treatments because they are resistant to many current anticancer therapies.[5] Therefore Gupta's discovery could be very important for cancer therapy in the future and it has inspired many researchers to do further studies and explore the biological properties of these compounds. On the basis of the above considerations, the development of novel polyether antibiotics and their derivatives as potential antitumour agents is very attractive. Thus, in this review the properties, mechanism of activity and potential applications of salinomycin and other polyether antibiotic in anticancer therapy will be discussed.

Chemical properties of polyether ionophores: The history of the polyether antibiotics started in 1951 when two compounds nigericin and lasalocid acid were isolated from different *Streptomyces* spp. Since then over 50 microorganisms have been found to produce carboxyl ionophores and over 120 structures have been reported for this class of compounds.[6] The term ionophore was first used in 1967 in reference to the ability of organic molecules to bind metal cations and form lipid soluble complexes that facilitate their transport across cellular membranes. Ionophores can diffuse from the extracellular space to the intracellular space, and back to the extracellular space or may remain in the plasma membrane as it transports metal ions between intracellular and extracellular spaces.[7]

Polyether antibiotics show a broad spectrum of biological activity ranging from antibacterial activity, especially against Gram-positive bacteria including also antibiotic-resistant *S. aureus* and *S. epidermidis* and other interesting activities such as antifungal, antiparasitic, antimalarial, antiviral, anti-inflammatory and tumour cell cytotoxic activity.[8]

Currently, seven carboxylic ionophores are marketed in the USA and around the world for use as anticoccidial drugs for poultry and growth promoters in ruminants. These include monensin (Coban, Rumensin, Coxidin), lasalocid (Avatec, Bovatec), salinomycin

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(Bio-cox, Sacox), narasin (Monteban, Maxiban), maduramycin (Cygro), laidlomycin (Cattlyst) and semduramycin (Aviax).[8] The structures of the most common polyether ionophores and others discussed here, are presented in Figure 1.

From the chemical point of view polyether antibiotics are molecules rich in oxygen atoms, that are present at many sites in a variety of functional groups. (Figs. 1–3). They always contain one carboxylic group, tetrahydropyran and tetrahydrofuran rings, several hydroxyl groups, and a ketone group.[9] These groups play a significant role in the process of coordination of a monovalent metal cation, for example, by monensin and salinomycin, and divalent metal cations for example, by lasalocid.

Recognition of different mechanisms of ion transport by ionophore antibiotics is needed to fully understand the mechanism of action of these compounds, including their anticancer activity. Knowledge of these mechanisms is very significant due to the biological activity of ionophores having the capacity to transport cations across cell membranes, thereby disturbing the natural Na^+/K^+ concentration gradient. The mechanism of transport of a cation by polyether ionophores is attributed to their ability to exchange protons and cations in an electroneutral process (Scheme 1).[7,9,10] It has been shown that the polyether ionophore anion (I^-) forms stable complexes with monovalent metal cations in which the coordination of the cations is always accompanied with a pseudo-cyclic structure stabilised by the 'head-to-tail' intramolecular hydrogen bonds between the carboxylate anion and hydroxyl groups (Figs. 2 and 3). In the electroneutral transport of the cations (M^+), the polyether ionophore anion ($I–COO^-$) binds the metal cation (with preference to Na^+ or K^+ cations) or proton (H^+) to give a neutral salt ($I–COO^-M^+$, Figs. 2b and 3b) or a neutral polyether ionophore in acidic form (I–COOH, Figs. 2a and 3a), respectively, and only uncharged molecules containing either the metal cation or proton can move through the cell membrane (Scheme 1a).[7,9,10] This mechanism is possible in the neutral or slightly alkaline environment of the cell because it is connected with the deprotonation of carboxyl group (COOH) leading to a carboxylate group (COO^-). The microenvironment of the tumour is significantly different; extracellular acidic pH is a common characteristic of human tumours while normal tissues generally have alkaline pH. Tumours are acidic due to their marked rate of lactic acid production because their cells maintain a high glycolytic rate even in conditions of adequate oxygen supply (aerobic glycolysis or 'Warburg effect').[11] The high anticancer activity of polyether antibiotics together with the known acidic conditions in tumour cells inspired Huczynski and

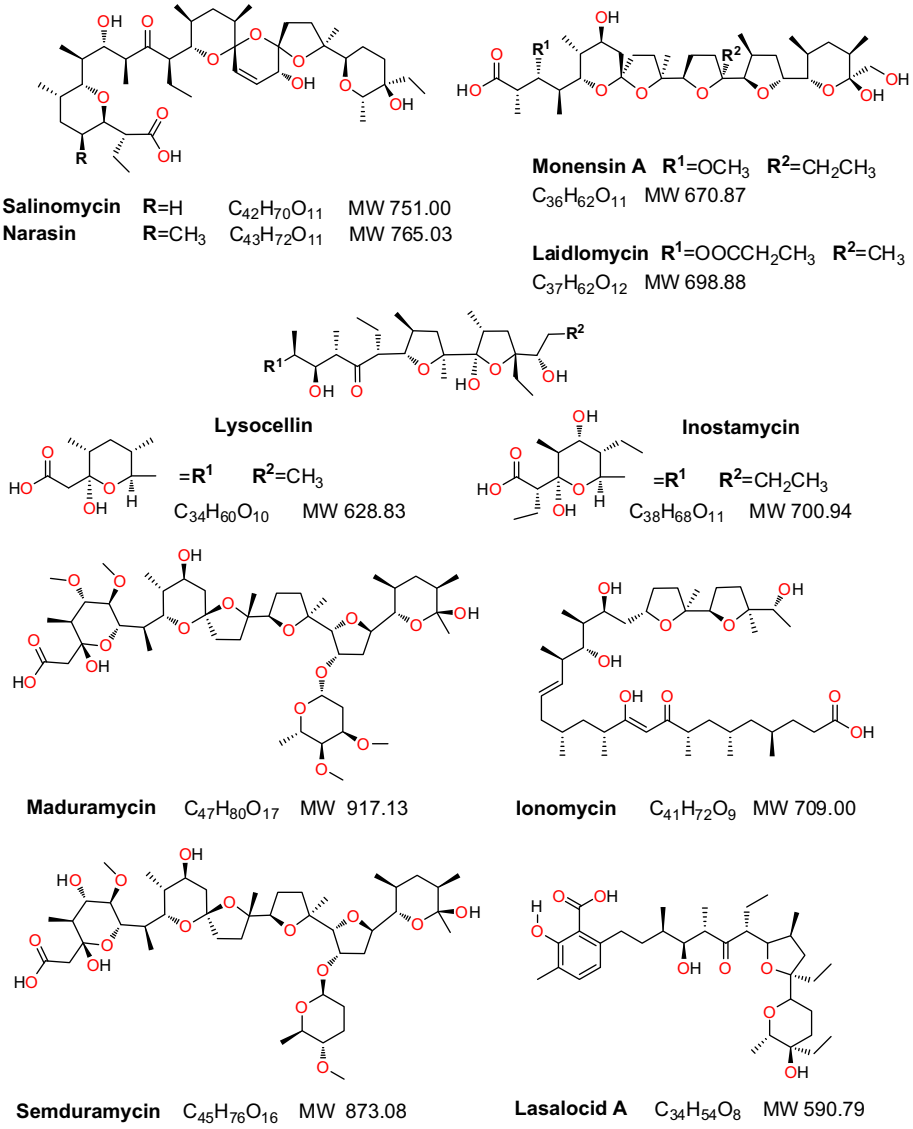


Figure 1. Structures of polyether antibiotics.

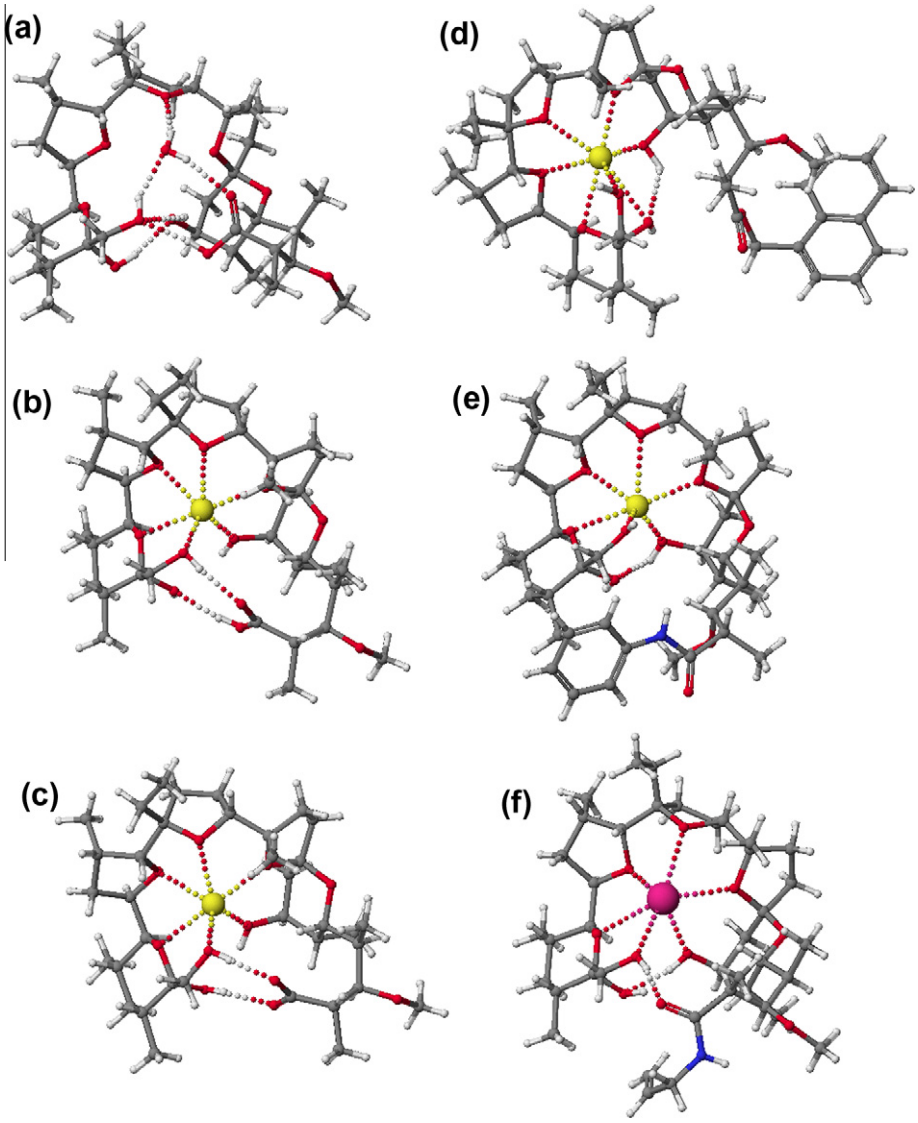


Figure 2. Structures of different types of polyether ionophore complexes presented on the exemplary crystal structures of monensin and its derivatives complexes (ball-and-stick presentation); (a) monensin acid hydrate;[12] (b) monensin acid complex with sodium chloride;[12] (c) monensin sodium salt complex[37], (d) complex of 1-naphthylmethyl ester of monensin A with sodium perchlorate;[37] (e) complex of *N*-phenylamide of monensin with sodium chloride;[37] (f) complex of *N*-allylamide of monensin with strontium perchlorate.[40] The solvent molecules and counterions are omitted for clarity.

co-workers to find a structural proof of the alternative mechanisms of transport of metal cations by polyether ionophores. (Scheme 1b).[12]

Their studies have shown that the process of cation complexation occurs in non alkaline environment. The complex is formed with the polyether ionophore in its acidic form (I–COOH–M^+, Figs. 2c and 3c) instead of the polyether ionophore anion (I–COO^-M^+, Figs. 2b and 3b) and the transport of cations is an electrogenic process (Scheme 1b).[12] Electrogenic or biomimetic transport mechanisms (Scheme 1) have been also postulated for derivatives of polyether ionophores with blocked carboxylic function such as amides and esters (Figs. 2c–e and 3c–e).

Having discussed the chemical principles of biological activity of polyether antibiotics the anticancer activity of these compounds will be clearer. Since salinomycin seems to be the most effective anticancer agent it will be the focus of the next section.

Salinomycin treatment in cancer stem cells and multidrug resistance cells (MDR): The history of discovery of anticancer activity of salinomycin is very interesting. In 2009 Piyush Gupta and co-workers tested a large number of 16,000 natural and artificial compounds for the ability to kill the stem cell–like cells. From this screening, 32 candidates were identified as active but only one of all tested compounds that is, polyether ionophore antibiotic—salinomycin, was proved to kill human cancer stem cells (CSCs) with more than 100-fold efficiency relative to that of the commonly used chemotherapeutic drug—paclitaxel (taxol).[4] These results became a milestone in the fight against CSCs because they are known to exhibit resistance to a broad spectrum of chemotherapeutic drugs, thereby surviving current cancer therapies and initiating long-term tumour recurrence, relapse and metastasis.[5] CSCs have been identified in a variety of human neoplasias, including cancers of the breast, brain, bone, skin, liver, bladder, prostate, colon and pancreas.[5] It has been also shown that salinomycin, contrary to paclitaxel, inhibits the ability of breast CSCs to form tumours in mice after being treated with 5 mg kg^{-1} salinomycin daily for five weeks. The reduction of the tumour mass and metastasis was accompanied by a reduced number of breast CSCs and increased epithelial differentiation.[5]

After Gupta et al. presented these important and innovative results concerning the anticancer properties and activity of

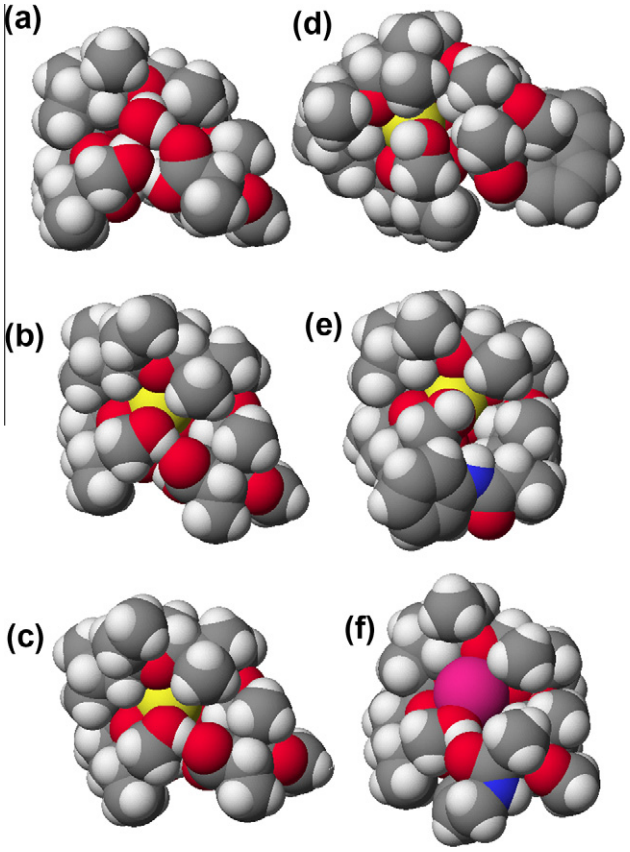
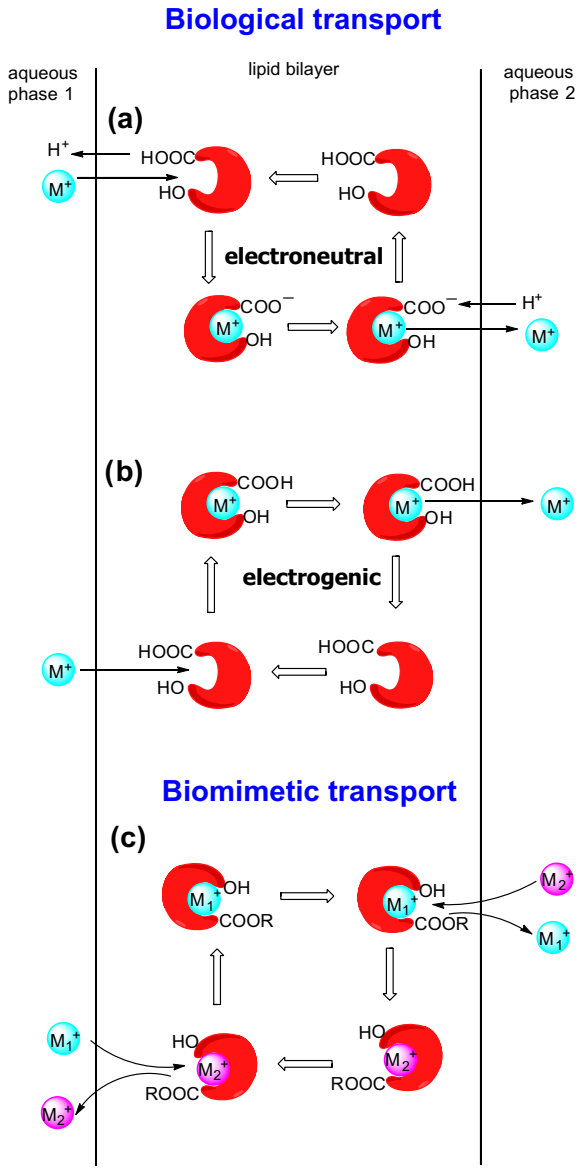


Figure 3. Space-filling models of the structures presented in Figure 2. The complexes of polyether ionophores have lipophilic surface and polar inner core containing oxygen atoms (red). The lipid soluble complexes of the polyether ionophores (Na^+ cation is yellow, Sr^{2+} cation is magenta) can be moved across lipid bilayer and cell membrane.

salinomycin,[4] it became viewed as a very promising anticancer drug candidate.[13]

The research group of Cord Naujokat from the University of Heidelberg has recently investigated the effects of salinomycin on different human cancer cells, including those that display anticancer drug resistance by different mechanisms. The results of these studies are very interesting because they show that salinomycin has induced massive apoptosis in human cancer cells of different origin, but not in normal cells such as human T lymphocytes.[14]

It has been shown that induction of apoptosis by different anticancer agents essentially depends on the expression of a functional (wild-type) p53 protein but what is worth noting is that salinomycin-induced apoptosis was independent of the p53 status of the cell. Different cancer cells were liable to massive apoptosis in response to exposure to salinomycin demonstrating that salinomycin can overcome apoptosis resistance due to overexpression and enhanced proteolytic activity of 26S proteasomes. These studies have shown clearly that salinomycin is able to induce apoptosis in cancer cells that exhibit resistance to apoptosis and anticancer agents by overexpression of Bcl-2, P-glycoprotein or 26S proteasomes with enhanced proteolytic activity. Salinomycin activates a distinct apoptotic pathway that is not accompanied by cell cycle arrest and that is independent of tumour suppressor protein p53, caspase activation, the CD95/CD95L system and the proteasome. Finally, the authors of these studies concluded that salinomycin should be considered as a novel and effective anticancer agent that overcomes multiple mechanisms of apoptosis resistance in human cancer cells.[14]



Scheme 1. Three mechanisms of the cation transport by the polyether ionophores. Due to their lipophilic surface, polar inner core containing oxygen atoms and one carboxylic group, the polyether ionophores are suitable to transport metal cations across lipid membranes. In the electroneutral transport (a), the metal cation is incorporated into the polyether skeleton of the pseudocyclic structure of ionophore, and electroneutral (I-COO^-M^+, Figs. 2c and 3c) complex diffuse to another interface, where deprotonated ionophore anion I-COO^- binds with H^+ to release M^+. The neutral ionophore acid molecule I-COOH (Figs. 2a and 3a), generated returns. In the electrogenic transport (b) the metal cation is bound and transported by ionophore acid molecule I-COOH forming I-COOH+M^+ complex (Figs. 2b and 3b). The biomimetic transport (c) can be realized by polyether ionophores with modified carboxylic group such as amides and esters (Figs. 2d–f and 3d–f).

Similar anticancer activity of salinomycin has been observed by Riccioni et al. who explored the effect of salinomycin on the proliferation and survival of different clinical multidrug resistance cells (MDRs) overexpressing P-gp.[15] Salinomycin elicited a dose-dependent inhibition of cell growth evident cells: a pronounced inhibitory effect on cell proliferation was evident at salinomycin concentrations of 1–5 μM. The authors have also evaluated the effect of salinomycin on induction of apoptosis of these cells. Salinomycin induced a moderate pro-apoptotic effect on cancer cells, particularly evident at days 2–3 of culture and at salinomycin dosages of 1–5 μM.[15] Riccioni et al. have performed experiments similar to those reported for

salinomycin and observed that another polyether antibiotic, nigericin, acts also as a very potent P-gp inhibitor, acting though a mechanism involving a change in P-gp conformation.[15]

In studies performed by Naujokat and co-workers the influence of salinomycin on leukemia stem cells, which are known to exhibit multidrug resistance by expression of ATP-binding cassette (ABC) transporters, was demonstrated.[16] One of the most important mechanisms of drug resistance in different cancer stem cells is the expression of ATP-binding cassette (ABC) transporters which belong to a highly conserved superfamily of transmembrane proteins capable of exporting a wide variety of chemotherapeutic drugs from the cytosol, thereby conferring multidrug resistance which is a major obstacle in the success of cancer chemotherapy.[17]

Therefore, studies showing salinomycin having activity on this type of leukaemia stem cells were important to verify that salinomycin can be regarded as a novel and effective agent for the elimination of leukaemia stem cells. It has been proved that salinomycin is able to overcome ABC transporter-mediated multidrug and apoptosis resistance in human leukaemia stem cell-like cells that exhibit resistance to a broad spectrum of chemotherapeutic drugs by virtue of expression of functional ABC transporters.[16] The uncommon apoptotic pathway[14] and the breakdown of ABC transporter-mediated multidrug and apoptosis resistance by salinomycin[16] may also contribute to the inability of leukemia stem cell-like cells to adapt to apoptosis-inducing concentrations of salinomycin.

Other studies described the ability of salinomycin to inhibit the Wnt signalling cascade. Because the Wnt/β-catenin signal transduction pathway plays a central role in stem cell development, and its aberrant activation can cause cancer drugs targeted at the Wnt/β-catenin pathway may be useful as anticancer agents.[18] It has been demonstrated that even nanomolar concentrations of salinomycin can inhibit Wnt1-induced signalling, but at this concentration the drug had very little effect on reporter gene activity in cells that overexpressed Fzd5/LRP6 or β-catenin. Much higher concentrations (4 μM) of salinomycin were needed to block the Wnt signalling induced by the downstream activators. Thus, salinomycin can suppress Wnt signalling by at least two different mechanisms, depending on drug dosage. It has been shown that the calcium polyether ionophore—ionomycin also strongly inhibited Wnt pathway activation by Fzd5/LRP6 at concentrations at which salinomycin was ineffective. Incubation of the malignant lymphocytes with ionomycin induced apoptosis within 48 h, with a mean IC_{50} of 230 nM. Under the same conditions, salinomycin failed to induce apoptosis in peripheral blood mononuclear cells (PBMCs) at a 100-fold higher concentration. This result suggests that salinomycin has selective cytotoxicity to CLL cells that are known to depend on Wnt signalling.[18]

Different studies have shown that salinomycin reduced the proportion of CD133+ cell subpopulations within colorectal cancers (CRC) which have been identified as cancer stem like cells (CSCs).[19] It is believed that many CRC therapies, that kill the bulk of cancer cells, inevitably fail because they do not eliminate colorectal CSCs, which survive to regenerate new tumours. Furthermore, salinomycin treatment decreased colony-forming ability and cell motility in human colon carcinoma cells (HT29). For the first time these studies have shown that salinomycin not only targets CRC stem cells specifically but also decreases the invasion and migration of CRC cells. Indeed, the latter effect seems especially attractive because more than 90% of cancer-related mortality arises from cancer invasion and metastasis.[19] Moreover it is important to emphasize that IC_{50} analysis has clearly demonstrated that CSCs are more resistant not only to paclitaxel[4] but also to other conventional anticancer drugs such as 5-fluorouracil (5-FU) and cis-diamminedichloroplatinum (cisplatin), but they are sensitive to salinomycin.[20]

Synergistic anticancer effect of salinomycin combined with anticancer drugs: Very recent studies performed by Zhi et al. and Kim et al. have demonstrated the ability of salinomycin to sensitize cancer cells treated by commonly used anticancer drugs such as paclitaxcel, docetaxcel, vinblastin, colchicines and also proved that salinomycin increased the sensitivity of cancer cells to the apoptotic effects of doxorubicin or etoposide.[20] It was shown that the ability of salinomycin to sensitize cancer cells to the effects of doxorubicin or etoposide is associated with an increase in DNA damage and a decrease in anti-apoptotic protein p21 levels. Next study has proved a novel mechanism of salinomycin sensitization in radiation-treated cancer cells by inducing G2 arrest and causing DNA damage and reduced p21 levels. Thus, it is clearly indicated that the mechanism underlying salinomycin sensitization is conserved in both chemo- and radiation-treated cells.[20]

Sensitization to the antimitotic drugs could be achieved with very low concentrations of salinomycin, suggesting that there is a possibility to minimize salinomycin toxicity associated with human cancer patient treatments. It has been shown that sensitization by salinomycin increased apoptosis and sensitized the cancer cells to antimitotic drugs by preventing G2 arrest, suggesting that salinomycin contributes to the induction of mitotic catastrophe. These results may contribute to the development of salinomycin-based chemotherapy for patients treated with antimitotic drugs.[20] The synergistic anticancer effect of salinomycin combined with gemcitabine in human pancreatic cancer cells has been also investigated. It is now well-documented that salinomycin inhibited the growth of CSCs,[4,13,16] while gemcitabine suppressed the viability of non-CSCs. Consistently, in vivo studies showed that salinomycin combined with gemcitabine could eliminate the engraftment of human pancreatic cancer more effectively than the individual agents. These data have indicated that administration of salinomycin, which targets CSCs, may constitute a potential therapeutic strategy for improving the efficacy of gemcitabine to eradicate pancreatic cancer.[21]

Effects of salinomycin on apoptosis: Recent studies performed on prostate cancer cells have evidenced that salinomycin induces apoptosis of these cancer cells by elevating oxidative stress through intracellular reactive oxygen species (ROS) production, which leads to the disruption of mitochondrial function and subsequent release of cytochrome c to the cytosol, and activation of the caspase zymogen cascade.[22] Apoptotic signals originating from mitochondria include a change in the electron transport system, loss of mitochondrial membrane potential (MMP, $\Delta\Psi_m$), failure of Ca^{2+} flux homeostasis, generation of ROS, and release of caspase activators. The link between ROS and apoptosis in salinomycin-exposed cells has been evident from the inhibition of apoptosis in pre-treated of cancer cells by antioxidant N-acetylcysteine. The study has also indicated that the chemo-resistance of hormone-independent cancer cells to salinomycin is higher than that of hormone-dependent cells, and compared to cancer cells, non-malignant prostate cells are relatively more resistant to salinomycin.[22]

In 2012 Ketola et al. demonstrated that salinomycin inhibits growth and migration of prostate cancer cells by reducing the expression of key prostate cancer oncogenes, inducing oxidative stress, decreasing the antioxidative capacity and cancer stem cell fraction.[23] Salinomycin was most effective in inhibiting vertebral-cancer of the prostate cells (VCaP) at half maximal effective concentration (EC_{50}) 380 nM. The authors also proved other interesting activity of salinomycin, for example, the fact that it reduced the migration of cancer cells. As the EC_{50} value of salinomycin in prostate carcinoma cells (PC-3) in response to 48-h exposure was higher than 1 μM, the salinomycin-induced anti-migratory effect is not due to inhibition of cell proliferation. The ability of salinomycin to inhibit prostate cancer cell growth and cancer stem cell population, without major effects on non-malignant prostate epithelial cells, is due to the induction of oxidative stress and the reduction of antioxidative properties. Thus, salinomycin and its derivatives may provide a novel selective approach to prostate cancer therapy.[24]

Ability of polyether ionophores to reverse multidrug resistance (MDR): Resistance to chemotherapy is a common clinical problem of patients with cancer. During treatment, tumour or neoplastic cells are often found to be refractory to a variety of drugs with different structures or modes of actions. Therefore, the search for new drugs is of vital importance. The numerous potential candidates reported so far include polyether ionophores. The specific effect of these compounds on cancer cells involves increasing their sensitivity to chemotherapy and the ability to reverse multidrug resistance (MDR) in human carcinoma. Nearly two decades ago the effect of several polyether antibiotics on colchicine resistance in human carcinoma multidrug-resistant KB-C410 cells, which exhibit about 4000-fold resistance to colchicine, was studied. The results of these studies demonstrated that 4 out of 14 polyether antibiotics are able to reverse colchicine resistance. Among them, laidlomycin was the most potent. It potentiated colchicine cytotoxicity on KB-C4 cells by about 700-fold at 1 μg/ml. The degree of potentiation was calculated by dividing the half maximal inhibitory concentration value (IC_{50}) of colchicines in the absence of the polyether antibiotic by the IC_{50} value of colchicine in the presence of the polyether antibiotic.[25]

Anticancer activity of inostamycin: Earlier studies have shown that another polyether antibiotic—inostamycin—can also reverse MDR in human carcinoma KB-C4 cells. The mechanism of its action was studied by the use of radioactively labelled vinblastine, a well known antimicrotubule drug used to treat certain kinds of cancers. Inostamycin dose-dependently increased the accumulation of vinblastine in multidrug-resistant KB-C4 cells at 0.5–2 μg/ml, while it did not enhance accumulation in the drug-sensitive KB-3-1 cells. At a concentration of 1 μg/ml inostamycin inhibited active vinblastine efflux from KB-C4 cells, but not from KB-3-1 cells, and inhibited vinblastine binding to KB-C4 membranes with an IC_{50} of 0.94 μg/ml Furthermore, vinblastine accumulated by treatment with 1 μg/ml of inostamycin was resistant to efflux from KB-C4 cells, even after the removal of inostamycin.[26]

The accumulation of vinblastine for 1 h in Kb-C4 cells increased about 4 times in the presence of inostamycin 1 μg/ml. The accumulation increased 3 times even after the cells had been pre-incubated with inostamycin for 30 min and then inostamycin was washed out. Detailed study has indicated that inostamycin irreversibly bound to KB plasma membranes, but the binding capacity did not parallel the amount of P-glycoprotein in three KB cell lines. Inostamycin was found to interact specifically with purified phosphatidylethanolamine. These results suggest that inostamycin can irreversibly inhibit P-glycoprotein by irreversibly binding to plasma membranes through phosphatidylethanolamine.[27]

The anticancer activity of inostamycin was very promising, therefore studies using this compound were continued. To establish whether the cytostatic effect of inostamycin is restricted to lung carcinoma cell lines or applicable to other type of cells, five oral squamous carcinoma (SCC) cell lines have been tested. Cell growth was suppressed by 62.5–125 ng/ml inostamycin, with non-viable cells being <1%, indicating inostamycin is cytostatic to SCC cell lines. Moreover, inostamycin induced an increase in G1/G0 cells (1.2- to 3.2-fold) over 24 h. These results suggest that inostamycin is a useful agent to prevent recurrences of tumour for oral SCC.[28]

On the other hand, it was shown that inostamycin is an inhibitor of cytidine 5′-diphosphate 1,2-diacyl-sn-glycerol (CDP-DG): inositol transferase. It significantly reduced epidermal growth factor (EGF)-induced in vitro invasion of the tongue carcinoma cell lines indicating that inostamycin would be useful for an anti-invasive agent in tongue cancer.[29]

It was also found that inostamycin increased the ability of paclitaxel (taxol) to induce apoptosis in Ms-1 cells.[30] A considerably higher concentration of paclitaxel was required for the induction of apoptosis in Ms-1 cells than in other cell lines tested. Treatment of Ms-1 cells with inostamycin, reduced the dosage of paclitaxel required to induce cell death by apoptosis. This effect of inostamycin is specific to Ms-1 cells and inostamycin did not increase the cytotoxicity of other antitumor drugs such as adriamycin, vinblastine, methotrexate, cisplatin, etoposide, or camptothecin in Ms-1 cells. Thus, inostamycin is a chemosensitizer of paclitaxel in small cell lung carcinoma Ms-1 cells.[31]

Furthermore, inostamycin abrogated the stimulatory effect of VEGF (vascular endothelial growth factor) on growth and migration activities of endothelial cells by targeting extracellular signal-regulated kinase-cyclin D1 and p38 pathways, respectively. Because inostamycin has antiproliferative and anti-invasive abilities, inhibition of phosphatidylinositol synthesis could be a potent therapeutic strategy against cancer as the *cancer dormant therapy*, that is persistence of residual tumor cells for long periods. Inostamycin, also induced activation of caspase-3(-like) proteases and apoptosis showing that it should be recognized as a potential anticancer drug.[32]

Anticancer activity of monensin: The antineoplastic effect of another polyether antibiotic, monensin, was also studied in depth. The antiproliferative effect of monensin on human lymphoma cell lines and SNU-C1 colon cancer cells, as well as NCI-H929 myeloma cells, showed that monensin significantly inhibited the proliferation of all the cell lines examined with a 50% inhibition in concentration of about 0.5 μM, and induced a G1 and/or a G2-M phase arrest in these cell lines.[33] Furthermore, monensin induced apoptosis in these cell lines. Detailed studies indicate that monensin inhibited the cell proliferation of human lymphoma cell lines by not only inducing cell cycle arrest, but also by triggering apoptosis through the loss of mitochondrial transmembrane potential ($MMP,_{m}$). Finally, these results suggest that monensin may be useful as a novel investigational drug for the treatment of lymphoma patients.[33]

Recently monensin has been shown to potentiate the in vitro cytotoxicity of immunotoxins, ribonuclease and overcome the drug resistance.[34] For example, these studies demonstrated that immunotoxin SWA11-ricin A chain could selectively eliminate almost 99.9% of clonogenic tumour cells at a concentration of 1×10^{-8} M and the cytotoxic activity of SWA11-ricin A chain was potentiated 100-fold in the presence of the carboxylic ionophore monensin at 1×10^{-7} M. Furthermore, the kinetic studies revealed that monensin two-fold enhanced the rate of protein synthesis inhibition and eliminated the lag phase suggesting a rapid effect on either the rate or route of internalisation.[34] It was also indicated that monensin is by itself a potent inhibitor of proliferation of both KB parent and KB/multidrug resistant (MDR) cells. In the presence of monensin, the ID_{50} of doxorubicin against KB/MDR cells after a 72 h drug exposure was about 5-fold, reduced while the presence of monensin did not significantly alter doxorubicin cytotoxicity against KB parent cells. In 1 h experiment, the presence of monensin by about two- to threefold increased the intracellular accumulation of doxorubicin in KB/MDR cells but not in KB parent cells. Monensin also markedly reduced doxorubicin efflux from KB/MDR cells. These results indicated that reversal of MDR by monensin may be due to facilitation of drug transport and subsequent enhancement of DNA damage in MDR cells.[34] Detailed studies proved that Monensin reduced drug efflux but did not alter subcellular distribution of daunorubicin, consistently with the view that monensin acts directly on P-glycoprotein in MDR cells.[34]

The lipophilicity and short half-life of monensin precludes its use and a suitable drug delivery system is needed to obtain the desired in vivo effects. Therefore, in several studies interesting delivery systems for monensin, such as long circulating liposomes and nanoparticles, have been developed. It has been shown that the delivery of monensin *via* long-circulating nanoparticles increases the in-vitro cytotoxicity of anticancer drugs and immunotoxins, as well as that long-circulating monensin liposomes

overcome the doxorubicin resistance in human breast adonocarcinoma (MCF-7/dox) cells.[35] The effects of monensin liposomes on drug resistance reversal, induction of apoptosis and expression of multidrug resistance (MDR) genes in a doxorubicin-resistant human breast tumour (MCF-7/dox) cell line have been evaluated. MCF-7/dox cells were treated with various anticancer drugs (doxorubicin, paclitaxel and etoposide) alone and in combination with monensin liposomes. The results of these studies indicated that monensin liposomes overcame drug resistance in MCF-7/dox cells to doxorubicin, etoposide and paclitaxel by 16.5, 5.6 and 2.8-times, respectively. The combination of doxorubicin (2.5 μg/mL) with monensin liposomes (20×10^{-8} M) induced apoptosis in approximately 40% cells, whereas doxorubicin (2.5 μg/mL) or monensin liposomes (20×10^{-8} M) alone produced minimal apoptosis (<10%) in MCF-7/dox cells. In conclusion, the result is that monensin liposomes potentiated the in vitro cytotoxicity of anticancer drugs in MCF-7/dox cells and offered an effective solution to overcome drug resistance in vitro. However, further in vivo studies are needed to confirm this in vitro findings.[35]

Griffin et al. studied the ability of monensin incorporated in unilamellar vesicles (liposomes) to potentiate antitumour immunotoxins in vitro and in vivo.[36] In their experiments monensin was incorporated into liposomes and used in combination with specific immunotoxins against human tumour cell lines in vitro (against H-MESO-1 malignant mesothelioma, LS174T colorectal carcinoma, and U373, U87, and MG-1 glioblastomas) and in vivo (mice were inoculated intra-peritoneally with H-MESO-1 cells).[36] These studies have shown that immunotoxin (specific ricin A-chain) plus 0.1 μM liposomal monensin was 5-fold more toxic for H-MESO-1 cells and 1000-fold and 2200-fold more toxic for human glioblastoma U373 and U87 cells respectively, than immunotoxin plus 0.1 μM free monensin in buffer. In vivo studies (in mice) liposomal monensin in combination with immunotoxin substantially prolonged survival, and three (21%) of 14 mice bearing H-MESO-1 xenografts treated with the liposomes showed no evidence of tumour on day 160 after treatment. Treatment with controlled immunotoxin plus liposomal monensin was ineffective.[36] These findings clearly suggest that encapsulation of monensin into liposomes increased the capacity of monensin to enhance the potency of cell-specific immunotoxin in vitro and in vivo.

The results of recent studies performed by Ketola et al. have indicated also that monensin is one of the most potent and cancer-specific inhibitors in a systematic sensitivity testing of most well known drugs and drug-like molecules in a panel of prostate cancer cell models.[37] The authors have screened 4910 known drugs and drug-like molecules in cancer cell lines to identify new prostate cancer cell growth selective inhibitors. The study indicated that only four compounds, including monensin, selectively inhibited cancer cell growth at nanomolar concentrations.[37] They proved that monensin effects at nanomolar concentrations are linked to induction of apoptosis and potent reduction of androgen receptor mRNA and protein in prostate cancer cells. Monensin also elevated intracellular oxidative stress in prostate cancer cells. Importantly, the antiproliferative effects of monensin were potentiated by combinatorial treatment with the antiandrogens. Taken together, the results suggest that monensin is a potential, well-tolerated, in vivo compatible drug with strong proapoptotic effects in prostate cancer cells, and synergistic effects with antiandrogens.[37] These studies suggest a general strategy in which the effects of antiandrogens could be enhanced by combinatorial administration with agents that increase oxidative stress in prostate cancer. The result of studies performed by Ketola et al. suggested that monensin,[37] as well as salinomycin,[22] should be recognized as new potential agents against prostate tumours.

Activity of polyether antibiotic derivatives: During the last decade, Huczyński et al. obtained many derivatives of two ionophores: monensin and lasalocid, and studied their structures and their complexes with metal cations and amines.[38] Recently, they also proved that some new polyether ionophore derivatives also show relatively high antibacterial activity, including against antibiotic-resistant *S. aureus* and *S. epidermidis*.[39] Modification of the carboxylic group of monensin leads to amides and esters which show very interesting and unexpected properties. For example, monensin methyl ester is able to form a proton channel which is thought to be able to transport protons through the cell membrane and is a suitable model for studying proton-transfer processes.[40] Moreover, new esters and amides of monensin are able to form stable complexes with divalent metal cations while unmodified monensin forms complexes only with the monovalent cation.[41] Modification of the carboxylic group of polyether ionophores should change the mechanism of ion transport through the cell membrane from electroneutral for acidic polyether ionophores to an electrogenic process for their amides and esters[12] which is important in view of the Warburg effect disused above.[11]

On the basis of this experience, and inspired by the high anticancer activity of salinomycin discovered by Gupta et al.[4] this group recently synthesized some amides of this antibiotic and evaluated their anticancer activity.[42] The antiproliferative activity of salinomycin and its derivatives were examined on four human cancer cell lines including vincristine-resistant and doxorubicin-resistant cell lines indicating that salinomycin and its derivatives break down drug resistance in the cancer cells. It is very important to note that almost all analogues of salinomycin showed stronger activity then unmodified salinomycin against drug resistant cell line what suggested breaking down drug-resistance of the tested cell line by these derivatives. These results show that salinomycin is a promising tool to reverse the MDR phenotype in clinic and to treat apoptosis resistant cancers and that modification of salinomycin can lead to more anticancer active compounds. Very recently this group also found that lasalocid and its complexes with amines are strong cytotoxic agents towards cancer cell lines. The cytostatic activity of these compounds is greater than that of cisplatin, indicating that lasalocid and its complexes are promising candidates for new anticancer drugs.[43]

Perspectives: Polyether ionophores are typical veterinary antibiotics and have never been used as antibiotic in humans, probably due to the considerable toxicity observed in mammals. In general, the marketed polyether ionophores have been found to be safe and effective in the target animal species within the approved dosage ranges.[44] However, overdosage, misuse and drug interactions have resulted in the ionophore toxic syndrome. Their toxicity is characterized clinically by anorexia, diarrhoea, dyspnoea, depression, ataxia, death, pathological changes and by degeneration and necrosis of heart and skeletal muscles. For example, the oral NOELs for monensin (no observable effect levels) were approximately 2–3.8 mg/kg in rats and 5 mg/kg/day in dogs after subchronic oral dosing of 3 months, 1.25 mg/kg/day in dogs after chronic oral dosing for 1 year, and 1.4 mg/kg/day in rats and 1.3 mg/kg/day in mice after chronic oral dosing for 2 years.[44]

No studies have been reported in which humans were intentionally exposed to polyether ionophores. Nonetheless, some of them have been tested in in vitro and in vivo animal models for the treatment of human malaria[45] and as sensitizers for immunotoxin and anticancer drugs.[21,36]

Assuming that the above uses of ionophore antibiotics would pass clinical trials and given regulatory approval, the potential benefits to humankind should far outweigh the risks of the low-level human exposures. It cannot be overemphasized that therapeutic doses for humans would be many folds lower than the single and repeated doses that are toxic for animals.

As discussed above, results of many studies have shown that both cancer stem cells (CSC) and multidrug-resistant cancer cells

(MDR) are effectively killed by polyether ionophores particularly by salinomycin. The investigation of polyether ionophores safety, toxicity, pharmacology and anticancer activity in humans is a challenge for the coming years. The chemotherapy approach is based on compounds that exert greater selective toxicity against cancer cells (including CSC and MDR cells) compared with that against other cells in the mammalian host. The activity of these compounds should be further studied in vitro to specify their mechanisms of action and in vivo to assess their activities and tolerance in the different types of cancer.

All the above discussed results show that polyether ionophores are currently well-recognized candidates to be clinically tested as anticancer drug candidates. The exact mechanism of anticancer activity of polyether antibiotics still has to be worked out. The studies performed so far have shown that these compounds affect cancer cells in a special way by increasing their sensitivity to chemotherapy (monensin, salinomycin, inostamycin) and reverse multidrug resistance (laidlomycin, monensin) in human carcinoma. Furthermore, these compounds have been found to be cytotoxic to the human carcinoma multidrug-resistant cells (monensin, salinomycin). Ionophore antibiotics also inhibit chemoresistant cancer cells by increasing apoptosis but up to now only salinomycin has been successfully able to kill human cancer stem cells (CSCs). Therefore, at present polyether antibiotics should be considered as new anti-cancer drugs for cancer chemoprevention and cancer therapy. I am convinced that the studies of the structure, chemical modification and anticancer activity of this group of compounds will increase in the near future and will provide more effective therapeutics for cancer treatment.

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