Ionophore Antibiotics: Toxicity, Mode of Action and Neurotoxic Aspect of Carboxylic Ionophores

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Abstract: Ionophores are antibiotics which are used as coccidiostat and growth promotant in veterinary practice. The ionophores make complexes with mono and divalent cations and facilitate the movement of metal ions by providing lipophilic channels thourough the hyrophobic lipid membranes. Use of ionophores are generally known to be safe and effective at the therapeutic doses in animal species. The most commonly used ionophores monensin, lasalocid and salinomicin are incorporated in the feed to prevent cocsidiosis and increase feed efficiency in poultry and cattle, respectively. However, accidental overdose, misuse, mixing errors and accidental ingestion in non-target species could result in toxicity in a number of animals. Horses, cattle, avian species, dogs, cats and rats are sensitive to ionophore toxicity. Toxic effects of ionophores are thought to be mediated by disrupting the normal ionic gradients of cells leading to mitochondrial damage, lack of cellular energy. A well-known toxic effect of ionophores is cardiac toxicity and muscle degeneration in suspected species. However, one less commonly known effect of ionophores is associated with nervous system leading to the neuropathy which is manifested with myelin degeneration and ataxia. The general toxic aspects, mechanism of toxicity as well as neurotoxic effect of ionophores are reviewed in this study.

Key words: Ionophores, neurotoxicity, toxicity, mechanism

INTRODUCTION

Improvement of feed efficiency and producing more lean protein with rapid growth at a lower cost has been primary objectives in animal husbandry. The most commonly used ionophores (Monensin, Lasalocid and Salinomycin) are incorporated into rations as anticoccidials in poultry and as growth promoters in ruminant feeding. The broad spectrum of antibacterial activity along with the ability of these compounds to passively transport cations across cell membranes is thought to be the basis of their mechanism of action in the prevention of coccidiosis and improving feed efficiency of ruminants (Elsasser, 1984). Increase in feed efficiency is thought to be associated with increasing ruminal propionic acid and decreasing acetate and butyrate with resultant improvement of energy and carbon retention in the rumen (Bergen and Bates, 1984). Use of lasalocid and monensin in cattle efficiently prevents lactic acidosis (Nagaraja *et al.*, 1981). The protective effect of ionophores against coccidia is associated with the ability of these drugs to transport ions across biological membranes and altering ionic balance in coccidia (Augustine *et al.*, 1992). However, ionophores could also affect the host adversely due to accidental overdose and misuse. Signs of ionophore toxicity comprise indefinite general symptoms. Most common signs of ionophore toxicity include anorexia, hypoactivity, leg weakness, ataxia, dyspnea and diarrhea. Reports indicate that horses, cattle, avian species, sheep, pig, dogs and cats are sensitive to ionophore toxicity (Galitzer and Oehme, 1984; Halvorson *et al.*, 1982; Hanson *et al.*, 1981; Oehme and Pickrell, 1999; Novilla, 1992; Van der Linde-Sipman *et al.*, 1999; Wilson, 1980).

In this study, the toxicity with emphasis to neurotoxic effect, general chemical and pharmacological aspects of most commonly used ionophores are reviewed.

Mode of action: Ionophores as a class are divided into 2 general groups based on mode of ion transfer across membranes. These include channel formers and ion carriers. Channel forming ionophores arrange themselves

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inside the membrane structure creating a hydrophilic channel for the ions. By this means, ions from outside the cell pass through the provided hydrophilic channel into the cell. This mode of ion transport is analogous to that of transport proteins found in cell membranes. A well-known example of this type of ion transport is carried out by gramicidin. To form a channel within the membrane, two gramicidin molecules are required to line up across the membrane. When 2 gramicidin molecules dimerize within the membrane, a hydrophilic channel is formed with outside consisting of hydrophobic residues (Becker *et al.*, 1996; Pressman, 1976). Ion carriers can be subdivided into neutral ionophores and carboxylic ionophores. Regardless of subdivisions, both neutral and carboxylic ionophores move the ions across lipid bilayer by diffusing together with ions. These ion carriers act in a way that they bind the ions on one side of the cell membrane and allow the ion to sit within the ion carrier. The resulting complex moves across the lipid bilayer and releases the ion on the other side of the cell membrane. Valinomycin, lasalocid, nigericin and A23187 are examples of ionophores with this mode of action (Becker *et al.*, 1996; Pressman, 1976).

Mechanism of toxicity of ionophores: The toxicity of ionophores has been widely studied in a number of animal species. A number of toxicity cases have been reported in horses, cattle, sheep, dog, cat, pig and avian species (Galitzer and Oehme, 1984; Halvorson *et al.*, 1982; Hanson *et al.*, 1981; Oehme and Pickrell, 1999; Novilla, 1992; Van der Linde-Sipman *et al.*, 1999; Wilson, 1980). Ionophores are generally safe and effective if used at recommended levels. However, ionophore toxicity might occur due to accidental overdose, misuse and mixing errors in the ration (Novilla, 1992).

The normal ionic gradient of the cell is maintained and tightly controlled by specialized transport complexes found in cell membranes. Examples of these complexes are Na^+-K^+-ATPase, Ca^{++}-Mg^{++}-ATPase and Na^+-Ca^{++} counter transport systems. If the cell membrane becomes permeable to ions which are normally controlled by these systems through ionophore mediated transport, the cells lose their ability to control and maintain physiologic ion gradients. Most of toxic effects of ionophores are thought to be mediated by disrupting the normal ionic gradients of cells (Elsasser, 1984). Alterations in cellular Ca^{++} concentration by lasalocid have been associated with disturbances in normal physiological function of the cardiac tissue due to dynamic regulation of cardiac muscle contractility by Ca^{++}. Ionophore cytotoxicity is thought to involve the influx of Na^+ and Ca^{++} ions with simultaneous efflux of K^+ ions leading to excess Ca^{++} overload within mitochondria, mitochondrial damage, lack of cellular

energy and ultimately muscle necrosis (Novilla, 1992). Another mechanism of Ca^{++}-mediated cell death occurs via apoptosis. It is thought that ionophores lead to apoptosis via calcium-activated endonucleases (Ojcius *et al.*, 1991). Furthermore, the mechanism leading to cell death is reported to be associated with activation of influx of Ca^{++} through NMDA receptor leading to activation of phospholipase A_2 and release of arachidonic acid (Safran *et al.*, 1996). Toxic effects on cardiac cells were also observed with monensin which typically affects Na^+ transport across the membranes. The basis for the cardiac effect of monensin is that movement of Na^+ ions inside the cell triggers Na^+/Ca^{++} exchange system leading to influx of Ca^{++} (Mollenhauer *et al.*, 1990).

The cardiac toxicity of some ionophores was studied in neonatal rat cardiac myocyte. Monensin induced cardiac myocyte cytotoxicity at concentrations = 10 mM *in vitro*. The effect caused by monensin on cardiac myocyte included "blebbing" of cell membranes and cellular swelling (Shier and Du Bourdieu, 1992). The elevated levels of Ca^{++} may affect numerous other cellular processes. The cellular processes activated by Ca^{++} include mostly intracellular signaling pathways and inference with important cellular enzyme systems including auto-oxidation of macromolecules, activation of phospholipase A_2 endonucleases and proteases with an end result of cytotoxicity (Tymianski and Tator, 1996).

Another effect of ionophores is seen on energy metabolism. Alteration of the cellular ionic gradient by ionophores can deplete intracellular ATP levels. This effect is much more detrimental in prokaryotic cells and accounts for its somewhat selective action. For example, monensin causes an influx of Na^+ ions leading to increased intracellular Na^+ concentration. The cell reacts by expending ATP to maintain normal Na^+ balance inside the cell. Subsequently, cells cannot meet the demand for ATP and lyse (Bergen and Bates, 1984). The reason for the selective action of ionophores on prokaryotes is based on differences on mode of energy usage and ability for osmoregulation. Coccidia are intracellular parasites that rely on the host cell for energy. Ionophores stimulate coccidia sporozoite's Na^+-K^+-ATPase as a consequence of ionic disturbance. However, rate of ion influx exceeds the capability of Na^+-K^+-ATPase pump to remove excess Na^+ ion because of depletion of energy sources. Increased intracellular Na^+ is followed by an influx of Cl^- to maintain electroneutrality. This in turn brings water from exterior causing swelling of the parasite. Since, the coccidia have no osmoregulatory organelles, they swell and burst (Smith and Galloway, 1983).

Effects of ionophore toxicity on nervous system: Alterations of nervous and muscle tissues were observed in ionophore induced toxicity. Lasalocid caused

Table 1: General aspects of toxicity of carboxylic ionophores in animal species

Animal species	Oral LD$_{50}$ (mg kg^{-1})	Clinical symptoms	Pathologic alterations
Horses Lasalocid-	Monensin-2-3 mg kg^{-1} 21.5 mg kg^{-1} Salinomycin- 0.6 mg kg^{-1}	Anorexia, colic, ataxia, tachycardia hypotension, dyspnea	Cardiac muscle damage Lung congestion, Kidney hyperemia
Dogs	Monensin- 20 mg kg^{-1}	Ataxia, mydriasis, Muscular weakness Myoglobunuria	Myopathy Muscle degeneration
Rats	Monensin- 35 mg kg^{-1} Lasalocid- 122 mg kg^{-1}	Tonic, clonic convulsions aggressive behaviours	Neuropathy, Demyelination Myelin splitting and edema in nerve
Cattle	Monensin- 22 mg kg^{-1}	Anorexia, incoordination ataxia, diarrhea, reduced cardiac function	Pulmonary edema Muscle hemorrhage Myocardial injury
Chickens	Monensin- 200 mg kg^{-1} Lasalocid- 71.5 mg kg^{-1}	Dyspnea, wing dropping gait abnormalities, ataxia reduced egg production and weight gain	Pale spleen, liver and lung congestion, cardiac and muscle damage, myelin edema and vacuolation in nervous tissue

Sources: Galitzer and Oehme (1984), Hanson *et al.* (1981), Novilla (1992), Oehme and Pickrell (1999), Osweiler (1996) and Wilson (1980)

membrane depolarization in rat muscle fibre membranes and induced spontaneous release of acetylcholine in phrenic nerve-diagram muscle preparation (Jansson *et al.*, 1976). Lasalocid also increased acetylcholine release from rat brain *in vitro* by an unknown mechanism (Richter, 1977).

It has been reported that lasalocid, monensin and ionomycin stimulate release of catecholamines from rat pheochromocytoma (Table 1) and this effect may be dependent on increasing intracellular Ca^{++} (Perlman *et al.*, 1980). Furthermore, lasalocid and ionophore A 23187 caused inhibition of fast axonal transport and decreased axonal microtubules with a concomitant increase in total calcium content of the nerve (Kanje and Hanson, 1981). One study showed that lasalocid caused selective degeneration of nerve cells *in vitro* by a mechanism dependent on increased Ca^{++} influx. This effect of lasalocid was blocked by the NMDA receptor blocker MK-801, suggesting possible involvement of excitatory amino acid in the neurotoxicity of Lasalocid (Safran *et al.*, 1996).

Alterations in nerve tissues and related clinical signs were reported in broiler chickens fed with Lasalocid. For example, lasalocid caused a dose dependent neurotoxicity in broiler chickens at doses greater than 11.25 mg kg^{-1}. The clinical manifestation of neurotoxicity resulted in ataxia. Nerve tissues from affected birds showed a number of changes in the histopathologic examination. These included myelin disruption, degeneration and vacuole formation in myelin (Gregory *et al.*, 1995; Roder, 1996). Salinomycin and monensin toxicity was reported in turkey breeders. The clinical condition in affected birds included

paralysis of legs leading to gait disturbances and abnormal positioning of head (Halvorson *et al.*, 1982). Contamination of cat food with salinomycin resulted in an outbreak of salinomycin neurotoxicity in cats. Toxicated cats developed paresis and paralysis of hindlimbs and lameness. Postmortem examination indicated a distal polyneuropathy including sensory and motor neurons. Although, both central and peripheral nerves were found to be affected, peripheral nerve injury was more severe in affected cats. Lesions were localized in axons, myelin sheath and the Schwann cells. These included destruction of myelin sheath with formation of digestion chambers, collapsed axonal sheath filled with foamy macrophages and swollen Schwann cells (Van der Linde-Sipman *et al.*, 1999). Rodent species may also be affected by ionophore neurotoxicity. Gad *et al.* (1985) reported that some ionophores including lasalocid cause neurobehavioral signs in mice and rats. Rats dosed with oral ionophores developed tremors, tonic-clonic convulsions and aggressive behaviors.

CONCLUSION

The reports clearly indicate that one of the toxic effects of ionophores is related to the nervous tissue and these effects are capable of inducing clinical and pathological changes in the nerves which can be called ionophore induced neurotoxicity. Although the cellular effect of ionophores is attributable to their ability to facilitate ion movements across cell membranes, the mechanism in neurotoxicity is not extensively studied and needs further investigations.

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