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Abiotic degradation of antibiotic ionophores

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ABSTRACT

Hydrolytic and photolytic degradation were investigated for the ionophore antibiotics lasalocid, monensin, salinomycin, and narasin. The hydrolysis study was carried out by dissolving the ionophores in solutions of pH 4, 7, and 9, followed by incubation at three temperatures of 6, 22, and 28 °C for maximum 34 days. Using LC–MS/MS for chemical analysis, lasalocid was not found to hydrolyse in any of the tested environments. Monensin, salinomycin, and narasin were all stable in neutral or alkaline solution but hydrolysed in the solution with a pH of 4. Half-lives at 25 °C were calculated to be 13, 0.6, and 0.7 days for monensin, salinomycin, and narasin, respectively.

Absorbance spectra from each compound indicated that only lasalocid is degraded by photolysis (half-life below 1 h) due to an absorbance maximum around 303 nm, and monensin, salinomycin, and narasin are resistant to direct photolysis because they absorb light of environmentally irrelevant wavelengths.

1. Introduction

Lasalocid (LAS), monensin (MON), salinomycin (SAL), and narasin (NAR) belong to the ionophore antibiotics. Ionophores are compounds with the ability to transport ions, mainly alkali and earth alkali metals, over the lipid bilayer of a cell membrane. Cells with uncontrolled influx or efflux of ions can spend so much energy on trying to restore the osmotic balance that they die. The polyether ionophores inhibit the activity of many bacteria, viruses, parasites, and eukaryotic cells (Khan et al., 2008). The toxicity of ionophores to human cells limits the current use of them to veterinary medicine.

The ionophores are naturally produced, bacterial toxins from genus *Streptomyces*. These carboxylic polyether compounds exist in several homologues, with the A homologue comprising the largest fraction produced (see Fig. 1).

Ionophores promote growth by manipulating the microbial flora in the intestines in most animal species as well as the rumen of ruminants (Page, 2003). This interaction results in improved metabolism, absorption, and digestion of an array of essential nutrients, including carbohydrates, amino acids, proteins, minerals and vitamins, and as a result, supplemented animals need less feed for the same nutrition (Page, 2003). Although countries like the USA and Australia allow their farmers to profit from the beneficial effects of ionophores as growth promoters (Cha et al., 2005; Page, 2003), the European Union only allows ionophores as medical treatment and not as growth promoters alone (VMD, 2011).

Besides antibiotic properties, the ionophores are also antiparasitics making them useful against coccidiosis. This is a parasitic disease resulting in diarrhoea, loss of weight and sometimes death due to inflammation in the small intestine (enteritis). Intensive animal rearing promotes conditions, in which the coccidia parasite can reach levels high enough for clinical symptoms to appear in the animals. Therefore, livestock are continuously fed with ionophores with a few days withdrawal time before slaughter. This prevents establishment (prophylactic treatment) or spread (metaphylactic treatment) of the disease within a flock or herd (VMD, 2011).

The degree of ionophore metabolism in animals is highly uncertain and not well investigated. As an example, chickens have been observed to excrete between 10 and 75% parent LAS depending on study (EFSA, 2004b, 2010a). The few conducted metabolism studies found for ionophores indicate that cattle excrete more of the parent compound than poultry do (Donoho, 1984; Elanco Products Company, 1986; Sassman and Lee, 2007).

Veterinary medicines enter the terrestrial environment mainly from intensively reared livestock when the manure or slurry is

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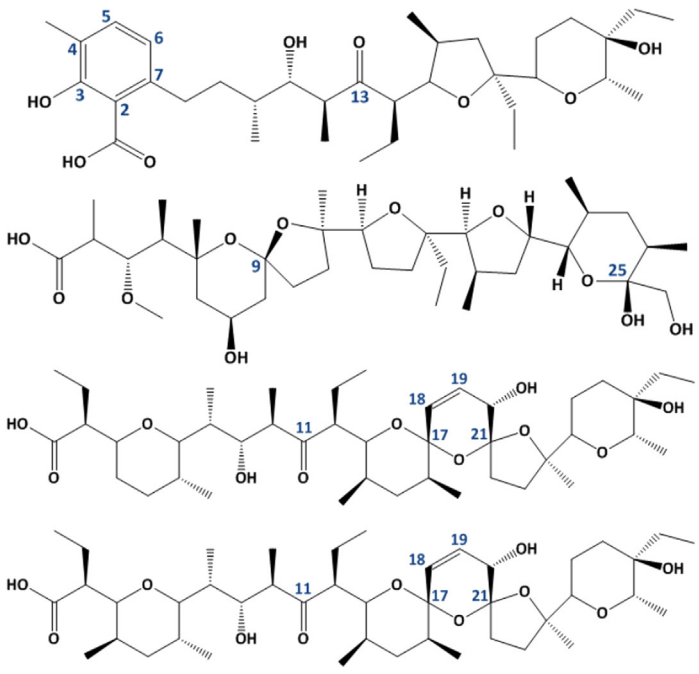

Lasalocid (LAS)

Aromatic ring between C2 and C7, ketone at C13.

Monensin (MON)

Ketal center at C9, hemiketal center at C25.

Salinomycin (SAL)

Ketone at C11, ketal centers at C17 and C21, double bond between C18 and C19.

Narasin (NAR)

Ketone at C11, ketal centers at C17 and C21, double bond between C18 and C19.

Fig. 1. Structures of the investigated ionophores. From top, the most common homologue structure of LAS, MON, SAL, and NAR. Adapted from Hansen et al. (2009a), Kim and Carlson (2006) and EFSA (2010b).

applied to land, but also through pasture-reared livestock, which excrete pharmaceutical residues directly into the environment as dung or urine (Boxall et al., 2004; Halling-Sørensen et al., 1998). From agricultural fields, drugs can leach out into surface and ground water if water soluble, otherwise they may adsorb to soil colloids and either accumulate or be transported within macropores (Davis et al., 2006). The ionophores are considered to be non-volatile (Elanco Products Company, 1986; Elanco Products Company, 1989) and no vapour pressure has been found in literature for any of them. Henry's law constant has been estimated to be in the range from $1.5 \cdot 10^{-18}$ to $2.1 \cdot 10^{-18}$ Pa L mol^{-1} (Thiele-Bruhn, 2003), which confirms the assumption that ionophores are non-volatile. Based on the physico-chemical properties (Table 1), it is assumed that ionophores are found in soil and sediment, where they will adsorb more as pH decreases. They will mainly be anionic in neutral and alkaline environments and could be transported with soil pore water to surface and ground water if the entire ionophore fraction does not adsorb to soil (Hansen et al., 2009a). If the ionophores adsorb heavily, they may instead be transported with soil colloids to pristine waters and then desorb. From water or soil, ionophores can be taken up in organisms, possibly causing adverse effects. One or more ionophores have been found in the environmental compartments predicted from the physico-chemical properties. Taking MON as an example, poultry litter has been found to contain 12 mg kg^{-1} (Furtula et al., 2010), agricultural soil 0.004–0.50 μg kg^{-1} (Song et al., 2010), sediment 31.5 μg kg^{-1} (Kim and Carlson, 2006), ground water 0.04–0.39 μg kg^{-1} (Watanabe et al., 2008) and surface water 6.2–1172 ng L^{-1} (Lissemore et al., 2006).

Generally, the current knowledge on dissipation in the environment is based on relatively few studies. Donoho (1984) showed that the 50% MON, which is excreted as parent in cattle manure, has a relatively long half-life ($t_{1/2}$) of around 88 days at 37 °C. Shorter half-lives were found for ionophore dissipation in poultry litter, ranging from 1.3 to 17 days (Dolliver et al., 2008; Ramaswamy et al., 2010; Schlüsener et al., 2006). The lower values were obtained for SAL at 20–25 °C and it is possible that many manure piles are situated in colder environments, thereby increasing the time until 50% dissipation.

The ionophores are generally resistant to hydrolysis (Elanco Products Company, 1989; Hansen et al., 2009b; Lissemore, 2005), even though a $t_{1/2}$ of around 3 days has been reported once for LAS at a pH of 9 (EFSA, 2010a), and once for NAR at a pH of 5 (Elanco Products Company, 1986).

Sunlight is not a fast dissipation process either, with $t_{1/2}$ estimates from 37.2 days to 55.1 days for MON, SAL, and NAR (Elanco Products Company, 1989; Hansen et al., 2009b; Hussain et al., 2012). An exception is a study by Elanco Products Company (1986), which found a $t_{1/2}$ for NAR of 1.5 days at pH 7.0. An absorbance spectrum for LAS obtained by Björklund et al. (2011) showed an absorbance maximum at 306 nm. This is within the solar wavelengths reaching the surface of the Earth and thus LAS might have a potential for direct photolysis. No spectra were identified in literature for the other compounds.

In summary, only little information is available about the environmental degradation of ionophores. In combination with the environmental findings, it is therefore possible that ionophores are persistent. Consequently, the aim of this study was to investigate if ionophores are susceptible to abiotic degradation by hydrolysis and/or photolysis.

2. Experimental

2.1. Chemicals and standard solutions

Lasalocid A sodium (LAS, purity 91%), monensin sodium (MON, purity 90%) and nigericin sodium (NIG, purity 98%) were purchased from Sigma–Aldrich (Steinheim, Germany). Salinomycin sodium salt 2.5 hydrate (SAL, purity 93%) was from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and narasin (NAR, purity 97%) was kindly donated by Eli Lilly (Indianapolis, IN, USA). Stock solutions were prepared in ethanol (EtOH) at a concentration level of 1.0 mg mL^{-1} and stored at −18 °C. NIG was used as an internal standard (IS) for the chemical LC–MS/MS analysis. A dilution of NIG to 4.0 μg mL^{-1} was made in EtOH, and 50 μL of this solution was spiked into each sample prior to sample preparation, resulting in a concentration of 0.2 μg mL^{-1}. A combined mixture containing 180 μg mL^{-1} of LAS, and 100 μg mL^{-1} of the ionophores MON, SAL, and NAR was prepared in EtOH, and dilution series for standard curves were made in the concentration range 0.01–2.00 μg mL^{-1}. A solvent mix was used for dissolving the sample in HPLC vials. It consisted of 1:99 water:acetonitrile and 10 mM formic acid and 50 μM sodium chloride. All utilized solvents (methanol, acetonitrile, heptane,