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Reduced persistence of the macrolide antibiotics erythromycin, clarithromycin and azithromycin in agricultural soil following several years of exposure in the field

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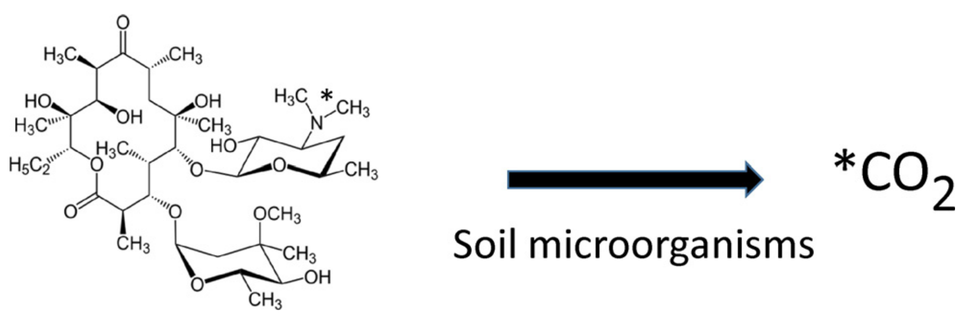
HIGHLIGHTS

- The impact of field exposure on persistence of macrolide antibiotics was evaluated.
- Soil samples were incubated in the laboratory with macrolides.
- Field exposure resulted in more rapid dissipation of all macrolides.
- Radiolabelled erythromycin and clarithromycin were rapidly mineralized.
- Macrolides were not carried over year-to-year in treated field soils.

GRAPHICAL ABSTRACT



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ABSTRACT

The macrolide antibiotics erythromycin, clarithromycin and azithromycin are very important in human and animal medicine, and can be entrained onto agricultural ground through application of sewage sludge or manures. In the present study, a series of replicated field plots were left untreated or received up to five annual spring applications of a mixture of three drugs to achieve a nominal concentration for each of 10 or 0.1 mg kg^{-1} soil; the latter an environmentally relevant concentration. Soil samples were incubated in the laboratory, and supplemented with antibiotics to establish the dissipation kinetics of erythromycin and clarithromycin using radioisotope methods, and azithromycin using HPLC-MS/MS. All three drugs were dissipated significantly more rapidly in soils with a history of field exposure to 10 mg kg^{-1} macrolides, and erythromycin and clarithromycin were also degraded more rapidly in field soil exposed to 0.1 mg kg^{-1} macrolides. Rapid mineralization of ^{14}C-labelled erythromycin and clarithromycin are consistent with biodegradation. Analysis of field soils revealed no carryover of parent compound from year to year. Azithromycin transformation products were detected consistent with removal of the desosamine and cladinose moieties. Overall, these results have revealed that following several years of exposure to macrolide antibiotics these are amenable to accelerated degradation. The potential accelerated degradation of these drugs in soils amended with manure and sewage sludge should be investigated as this phenomenon would attenuate environmental exposure and selection pressure for clinically relevant resistance.

1. Introduction

Fertilization of ground for crop production with beneficial organic amendments is an extremely important and widespread agricultural

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practice. Notably, amendment with manures from livestock or poultry production systems increases soil organic matter content, and recycles nitrogen, phosphorus and other nutrients that would otherwise have to be provided through chemical fertilizers at greater cost to the farmer. In many jurisdictions in North America, Europe and elsewhere, municipal biosolids (*i.e.* treated sewage sludge) are also approved as an amendment for agricultural land (O'Connor et al., 2005). Both manures and biosolids will contain enteric pathogens, as well as various organic and inorganic contaminants. The potential risk from these biological and chemical contaminants to environmental quality and human health must be managed through judicious practice such as pre-application digestion or composting, or offset times between application and the harvest of crops for human consumption (Mafor Expertise scientifique collective, 2014).

Organic chemicals that persist during the sewage treatment process and that partition into the sludge will be entrained onto agricultural land through the application of biosolids. Biosolids typically carry a wide variety of pharmaceuticals and personal care products at concentrations ranging from mg to μg per kg dry weight (McClellan and Halden, 2010; Verlicchi et al., 2015)). Pharmaceuticals and personal care products that are broadly biocidal, antibacterial (antibiotic), and antifungal are detected in soils receiving biosolids, and also in leachate or runoff from fields receiving biosolids (Gottschall et al., 2013; Sabourin et al., 2009; Walters et al., 2010). Likewise, animal and poultry manures can contain parasiticides and antibiotics that are excreted largely unchanged, and have the potential to move from the point of application to adjacent surface or shallow groundwater (Blackwell et al., 2009; Pope et al., 2009).

There is a concern that environmental emissions of antibiotics *via* land application of wastes from medicated animals or people, effluents from pharmaceutical manufacturing or municipal wastewater treatment, or irrigation with reclaimed water may promote increased antibiotic resistance in environmental bacteria (Berendonk et al., 2015; Gatica and Cytryn, 2013; Gaze et al., 2013; Pruden et al., 2013). A reservoir of antibiotic resistance genes in the environment that is made larger through agricultural practice may represent an enhanced threat to human health *via* ingestion of contaminated food or water (Ashbolt et al., 2013; Wright, 2010). Furthermore, there is concern that should environmental concentrations of antibiotics be sufficient to inhibit prokaryotic microorganisms, the key ecosystem services they provide could be compromised (Brandt et al., 2015).

Within this context, we have been evaluating the potential long term interactions of pharmaceuticals with soil microorganisms in field experiments undertaken in London, Ontario, Canada. Annually at spring time, a series of replicated plots is amended with environmentally relevant or excessive concentrations of pharmaceuticals carried in animal or human wastes (Konopka et al., 2014; Topp et al., 2013). Various pharmaceuticals are added directly to soil to simulate exposure through an annual amendment at the start of the crop growing season. The absence of an organic amendment matrix simplifies the interpretation of the results, at the expense of a more realistic exposure scenario. In the present study, a series of replicated plots received annual applications of a mixture of the macrolide antibiotics erythromycin, clarithromycin and azithromycin. These drugs are widely used in human medicine and thus could reach agricultural land through the application of biosolids or wastewater. In Canada, erythromycin is approved for veterinary use for various food animals including beef cattle, poultry, sheep and swine (http://www.hc-sc.gc.ca/dhp-mps/vet/mrl-lmr/mrl-lmr_versus_new-nouveau-eng.php; accessed 26.1.2016). Approximately 2 tonnes of erythromycin, 3.5 tonnes of azithromycin and 17 tonnes of clarithromycin are sold annually for human use in Canada (McLaughlin and Belknap, 2008) Anaerobically digested municipal biosolids used in crop drug uptake studies carried 67.4 μg kg^{-1} dry weight clarithromycin and 213 μg kg^{-1} dry weight azithromycin (Sabourin et al., 2012). A survey of biosolids from several municipalities in Michigan USA detected erythromycin at concentrations ranging from undetectable to 62.8 ± 5.8 μg kg^{-1} dry weight (Ding et al., 2011). About 60% of erythromycin and 25% of the azithromycin entering a municipal wastewater plant servicing about 1.5 million people in Southwestern China was estimated to be released in effluent (Yan et al., 2014). Taken together, environmental exposure to macrolides *via* wastewater effluent and land application of biosolids will certainly occur.

A key observation from a study undertaken in London Canada with antibiotics commonly used in swine production was that the sulfonamide sulfamethazine and the macrolide tylosin were dissipated much more rapidly in soils with a history of annual exposure to the antibiotics relative to "naïve" control soil that was not exposed to the antibiotics (Topp et al., 2013). Similarly, three years following treatment of a German soil with swine manure containing ^{14}C-labelled sulfadiazine, radiolabelled drug residues were mineralized to $^{14}CO_2$ much more rapidly than in soil that had not been treated with swine manure (Tappe et al., 2013). A *Microbacterium* sp. was independently isolated from these two studies that grew at the expense of sulfonamide drugs, mineralizing the phenyl portion and excreting the heterocyclic portion as an end product of metabolism (Tappe et al., 2013; Topp et al., 2013). A precipitous decline in soil persistence associated with a history of exposure to a chemical has been called accelerated biodegradation, and has been observed for several pesticide classes following multiple repeated applications to soil (Chapman and Harris, 1990). If generalized with antimicrobial medicines in agricultural soils, this phenomenon would reduce their environmental persistence, reducing selection pressure for the development of resistance of clinical significance.

Within this context, the present study evaluated the impact of repeated soil exposure to a mixture of macrolide antibiotics on the kinetics and pathways of macrolide dissipation in soil. The specific objectives were 1. Determine if exposure to environmentally reasonable (0.1 mg kg^{-1}) or unreasonably excessive (10 mg kg^{-1}) concentration of the macrolides influenced the kinetics of dissipation relative to naïve soil; 2. On the basis of disposition of ^{14}C mass balance, and HPLC tandem-MS analysis of extractable transformation products, comment on the dissipation pathways of the antibiotics; and 3. Determine if the laboratory-derived dissipation kinetics were coherent with persistence rates *in situ*.

2. Materials and methods

2.1. Chemicals

The structures for the three macrolide antibiotics used in the present study are presented in Fig. 1, and their key properties in Table 1. Erythromycin, clarithromycin, and azithromycin were purchased from Sigma Chemical Co. (Toronto, ON). Erythromycin-[*N*-methyl-^{14}C] (radioactive purity >99%, specific activity 2.035 GBq $mmol^{-1}$) was purchased from American Radiolabeled Chemicals Inc. (St-Louis, MO) and clarithromycin-[6-*O*-methyl-^{14}C] (radioactive purity >99.8%, specific activity 1.965 GBq $mmol^{-1}$) was purchased from Moravek Inc. (Brea, CA). Stock solutions of labelled (16.67 Bq μL^{-1}) and unlabelled 1 mg mL^{-1} antibiotics were prepared separately in ethanol (final radioactive concentration of labelled and unlabelled) and stored at −20 °C until used in experiments.

2.2. Field experiment

The experiment was undertaken at the Agriculture and Agri-Food Canada experimental farm in London, ON, Canada. The soil is a silt loam (grey brown Luvisol) with the following properties: pH of 7.5, cation exchange capacity of 13.2, sand/silt/clay (%) of 18/67/15, and organic matter content of 3.4%. Prior to the experiment the soil had not been treated with manure or biosolids, materials that could have carried drugs. Twelve 2 m^2 plots isolated by fiberglass frames were established in the spring of 2010. The plots received an annual spring application of a mixture of erythromycin, clarithromycin and azithromycin at one