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Abstract

We investigated the fate of the drug ivermectin in the soil. We found that ivermectin was transformed solely by photos, leading to the formation of two ivermectin isomers. We indeed failed to detect any biotransformation reaction of the chemical either in the soil or in fungal liquid cultures. According to its limited water solubility, the bioavailability of ivermectin was very low in the soil solution. Here, we show that ivermectin, transferred to the soil from faeces of drug-treated cattle, could be stored for long periods in the soil.

Keywords

Avermectin; Ivermectin; Soil; Transformation; Bioavailability

Introduction

Avermectins are a class of macrocyclic lactone drugs with insecticidal and anthelmintic properties that have been developed for the protection of animals (Campbell 1985), humans (Dadzie et al. 1987) and crops (Putter et al. 1981). Examples of avermectins used for cattle protection include ivermectin, doramectin, eprinomectin and moxidectin. A recent formulation, the intraruminal-sustained release bolus, is considered as an efficient means to control endoparasitic nematodes in ruminants because of the long-term protection of animals. 80–90% of the drug dose administered by the bolus is excreted without transformation in faeces during a 4-month period (Alvinerie et al. 1999). Faecal excretion of ivermectin induces toxic effects on non-target organisms, including key

dung-colonising families of insects. The drug has also been shown to negatively influence the decomposition of dung organic matter in soil (Sommer et al. 2002), but data are lacking concerning its impact on soil inhabiting (micro)-organisms.

The ecotoxicological risk assessment of bolus on pasture-land ecosystems has not been performed in detail in the past. Our aim is to provide data concerning possible side effects of high levels of residues deposited in the soil. In this paper, we report the results concerning the fate of ivermectin in soils, as a basis for exposure assessment. These data will make it possible to design impact studies of ivermectin on soil fungi. The impact studies will be presented in a further paper.

Experimental

Chemicals

High-purity ivermectin (22,23-dihydroavermectin B1) and chemical reagents were purchased from Sigma-Aldrich. 8,9- and 10,11-Z isomers of ivermectin were obtained by photo-transformation of a drug solution layered on glass plates under sunlight, then identified using mass spectrometry, ^{1}H and ^{13}C NMR, according to Mrozik et al. (1988).

Soil characteristics

The soil used in this study was a silt loam collected in the 10–20 cm layer of a meadow in Versailles, France. It contains 25.5% sand, 55.0% silt and 19.5% clay, and its organic matter content was 1.65%. The soil pH was 8.1 and the cationic exchange capacity was 10.2 meq 100 g^{-1}. The soil was 2 mm sieved then used immediately.

Degradation experiments in soils

Incubations were performed on 25 g dry weight soil samples spiked with 25 µg ivermectin (1 mg kg^{-1}). The soil was kept at 80% of its moisture-holding capacity by adding water. Soil samples were incubated either in sterile or in non-sterile conditions in the dark at 25 °C, or under sunlight at ambient temperature of June, with a mean value of 20 °C).

Bioavailability of ivermectin

The amount of ivermectin bioavailable for soil fungi was determined using the protocol described by Gaillardon and Dur (1995). Soil samples (10 g equivalent dry matter) were placed in 5-cm diameter Petri dishes to give a 3-4 mm thick layer. Acetonic solutions of ivermectin were applied to the surface of the soil to obtain a final concentration of 1 mg kg^{-1}. The solvent was allowed to evaporate for 30 min and water was added to ensure that 80% of the moisture-holding capacity of the soil was maintained. Concentrations of ivermectin in the soil solution were determined 24 h after treatment. Two superposed 42.5-mm-diameter glass microfibre filters GF/A (Whatman) were laid on the soil surface and slight pressure was applied for 10 s to favour wetting of the filters. The upper filter was then recovered, weighed to determine the volume of soil solution, and dried overnight at 40 °C.

Degradation experiments in fungal liquid cultures

The ability of two strains of filamentous fungi, *Trametes versicolor* (ATCC 32745) and *Fusarium solani* (isolated from the soil) was evaluated in liquid cultures. They were grown on a culture medium (10 mL in 150-mL Erlen- meyer flasks, Lesage-Meesen et al. 1996) with maltose and ammonium tartrate as carbon and nitrogen sources, inoculated with a mycelial mat on agar plug (10-mm diameter). Cultivation was carried out statically in the dark at 25 °C for 2 days. Then, the medium was supplemented with 1 mg L^{-1} ivermectin. Drug transformation was then checked after 8 days of growth.

Analytical methods

During the soil degradation experiments, residual ivermectin and possible transformation products were extracted every week by shaking the soil (25 g dry weight) with 50 mL acetonitrile for 1 h. The organic extract was then centrifuged (5 min, 2,000 g), and aliquots of 6 mL were mixed with 9 mL distilled water; 10 mL of the solution was concentrated on an MCH-10 C18 guard column (30x4.6 mm; Varian) with an isocratic high performance liquid chromatography (HPLC) pump at a flow rate of 1 mL min^{-1}. The guard column was fixed to a ten-way valve. The extracted compounds were then eluted on a C18 Nova-Pak analytical column (150 x 4.6 mm; Waters) with an HPLC

pump delivering a solvent system composed of H3PO4 (0.1% in water), acetonitrile and methanol (20:57:23, v/v/v). The column eluate was monitored at 245 nm using a variable wavelength UV-Vis detector. The detection limit was 80 ng ivermectin.

To analyse bioavailability assays, dry filters were extracted with 10 mL acetonitrile. The eluate was then evaporated to dryness and the dry residue was subjected to the derivation procedure already published (Alvinerie et al. 1999). Briefly, the dry residue was dissolved in 100 µL of an N-methylimidazole solution in acetonitrile. To initiate the derivatisation, 150 µL trifluoroacetic anhydride solution in acetonitrile was added. After derivatisation, 100 µL of the solution was analysed hy HPLC. A mobile phase of H_3PO_4 (0.1% in water), acetonitrile and methanol (4:66:30, v/v/v) was used. Fluorescence was detected at an excitation wavelength level of 377 nm and an emission wavelength of 427 nm. The detection limit was 1 ng derivatised ivermectin.

Ivermectin in fungal cultures was analysed using the following procedure: Fungal biomass (700-800 mg dry weight) from an Erlenmeyer flask was separated from the medium by filtration on a glass fiber filter after 8 days. The biomass was then heated in the presence of MeOH (20 mL per Erlenmeyer flask) at 70 °C for 10 min. After cooling, each extract was concentrated under vacuum and dissolved in 20 mL diethyl ether. The organic extract was then pooled with the corresponding culture medium and shaken for 2 min in a separatory funnel. Finally, the organic fraction was recovered and dried on $MgSO_4$. These steps were performed in triplicate. The resulting organic extracts were pooled, evaporated to dryness and dissolved in 1 mL MeOH for HPLC analysis without derivatisation. Each experiment was done in triplicate. Results are expressed as means. The standard deviation was less than 10% of the mean.

Results and discussion

Transformation of ivermectin in the soil

The fate of ivermectin in soil was studied under various incubation conditions (Fig. 1). The initial amount of the ivermectin added (1 mg kg^{-1}) is commonly found in the faeces of calves treated with the bolus (Alvinerie et al. 1999), and may represent the maximal amount of ivermectin in the subsurface of the soil.

The results show that under sterile conditions in the dark, the drug ivermectin was not degraded (Fig. 1). Further, under non-sterile conditions, only a slight decrease of ivermectin concentrations

was observed, as a possible biological effect of endogenous microflora. A half-life of the chemical of around 240 days was calculated under these conditions.
By sharp contrast, exposing the soil samples to sunlight induced in a rapid decrease of ivermecin amounts extracted from the soil, which was stimulated by the mixing of the soil. The corresponding half-life of the chemical was 21 days. These results are consistent with the data previously published (Halley et al. 1990).
Two minor peaks from extracts obtained from soils incubated under sunlight were detected on the HPLC chromatograms. They co-eluted with standards of 8,9- and 10,11-Z isomers of the drug formed through photo- transformation (Fig. 2), but their low amounts prevented any structural determination. Formation of these com- pounds has also been reported previously (Mrozik et al. 1988). No other transformation products could be identified in organic extracts.

Bioavailability of ivermectin in soil

We measured the amounts of ivermectin in the soil solution after a 24-h period of sorption of the chemical onto soil samples. The ivermectin measured by HPLC amounted to 23.8±13.8 ng (mean ± SD of three replicates) per filter, and corresponded to a concentration of 6 µg L^{-1} soil solution. This value fits with the aqueous solubility of the highly hydrophobic ivermectin, which could be approximated to 10^{-8} M for the design of toxicity experiments on soil micro-organisms.
The soil used in this study exhibited a moderate organic carbon content, responsible for the adsorption of ivermectin, a neutral compound. As a consequence, the bioavailability of the drug appears essentially governed by the organic carbon content of the soil rather than its other properties.

Biotransformation of ivermectin in fungal liquid cultures

We studied the biotransformation of the drug in liquid cultures of *T. versicolor* and *F. solani* (Table 1). The ability of these strains to transform xenobiotics has been extensively studied in the laboratory.
After 8 days, ivermectin concentration in the medium decreased to 70.4% of the initial amount added in controls flasks non-inoculated with either strain. This decrease was a result of adsorption of the chemical on glass surfaces. Ivermectin amounts found after the same period in the

Erlenmeyers flasks inoculated with of *T. versicolor* or *F. solani* were slightly higher, evidencing the lack of transformation of the drug by the fungal strains.

T. versicolor is a white-rot basidiomycete producing exocellular oxidases, namely laccases, involved in the transformation of natural or xenobiotic compounds. Spiking the cultures with 20 mM xylidine, a well-known inducer of laccase production (Mougin et al. 2002), did not enhance ivermectin transformation.

In order to increase the uptake of ivermectin by *F. solani*, a fungal strain transforming xenobiotics using intracellular enzymes, several cultures were treated with neutral surfactants, namely Triton X100, Tween 20 and Brij 35, each at two-fold its critical micellar concentration. As the concentrations of ivermectin globally increased in the treated cultures, we concluded that the presence of the surfactants did not have an enhancing effect on ivermectin transformation by the fungus.

Conclusion

Our data show that that ivermectin can be stored for long periods in the soil in dark conditions. We were unable to detect any biotransformation of ivermectin by filamentous fungi, including a powerful white-rot strain. Bioavailability of the drug for soil inhabiting organisms was extremely low in the soil solution, but it may be increased according to the exposure routes. For example, ivermectin associated with the surface of particulate matter could penetrate the gastrointestinal tract of animals. Ivermectin could also represent a risk for organisms living in aquatic ecosystems that can be contaminated by the drug strongly adsorbed onto soil particles or suspended materials.

We know the concentration of the drug in the soil solution and its main chemical form, the parent com- pound. It is therefore now possible to design impact studies of ivermectin on soil fungi.

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Table 1. Ivermectin extracted from 8-day liquid cultures submitted to various incubation conditions

Incubation conditions	Extracted ivermectin (% of initial amount)
Non inoculated control	70.4
T. versicolor alone	81.0
T. versicolor + xylidine	79.7
F. solani alone	74.0
F. solani + Triton X100	71.5
F. solani + Tween 20	80.5
F. solani +Brij 35	84.5

Fig. 1. Transformation of ivermectin in soil under various incubation conditions

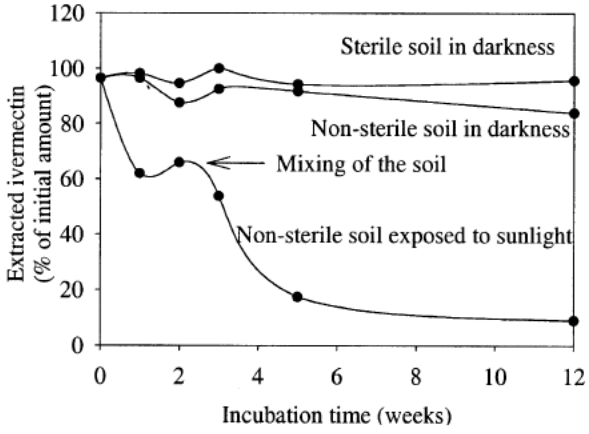

Fig. 2. Photo-isomerization of ivermectin