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Enantioselective degradation kinetics of metalaxyl in rabbits

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Abstract

Metalaxyl [methyl-*N*-(2′-methoxyacetyl)-*N*-(2,6-dimethylphenyl)-D,L- alaninate] is a potent phenylamide fungicide. The (−)-(*R*)-isomer accounts for most of the fungicidal activity. A possible stereo and/or enantioselective kinetics of metalaxyl in rabbits was investigated by intravenous injection. The concentrations of (−)-(*R*)- and (+)-(*S*)-metalaxyl in plasma, liver, and kidney tissue were determined by HPLC with a cellulose–Tris-(3,5-dimethylphenylcarbamate)-based chiral stationary phase and gas chromatography-mass spectroscopy. After intravenous administration of racemic metalaxyl (40 mg/kg), the (+)-(*S*)-enantiomer levels in plasma, liver, and kidney decreased more rapidly than the (−)-(*R*)-isomer. The area ratio of the (−)-(*R*)-/(+)-(*S*)-enantiomer under the concentration–time curve ($AUC_{0\rightarrow\infty}$) in plasma after drug application was 1.62. The total plasma clearance value of the (+)-(*S*)-enantiomer was 1.53 and higher than that of the (−)-(*R*)-enantiomer. The [*R*]/[*S*] ratio in plasma was >1 for standard rac-metalaxyl at each time point. The other pharmacokinetic parameters of the enantiomers were also different. The results indicate substantial stereoselectivity in the degradation of metalaxyl enantiomers in rabbits.

Keywords: Enantioselective degradation kinetics; Chiral HPLC analysis; Metalaxyl; Rabbit

1. Introduction

A large number of organic agrochemicals are chiral compounds and consist of mixtures of stereoisomers or enantiomers having different biological activity and physiological properties. In some cases, the biological activity of a pesticide may be attributed to one stereoisomer with the other isomer scarcely active at all. Alternatively, the enantiomers may have similar biological activity or one enantiomer may produce a completely different biological response than its antipode [1]. Many chiral pesticides are released into the environment as racemic mixtures of enantiomers. Thus, in recent years, growing concern about the side effects of chiral agrochemicals on nontarget organisms and natural resources has promoted the use of enantiopure or stereochemically enriched compounds. It is important to point out that stereochemistry strongly influences not only biological activity but also processes such as deg-

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radation and metabolic behavior in organisms and in the environment. Pesticide stereoselective kinetic or degradation studies make important contributions in improving pesticide safety to humans and animals and in minimizing contamination of the environment.

Metalaxyl [methyl-*N*-(2′-methoxyacetyl)-*N*-(2,6-dimethylphenyl)-D,L-alaninate] is an important phenylamide fungicide. Introduced in 1977, it is widely used for control of plant diseases caused by *Phythophthora infestans* and *Pythium ultimum* [2,3]. Metalaxyl interacts with the RNA polymerase-I-template complex, inhibiting incorporation of ribonucleotide triphosphates into ribosomal RNA [4]. Metalaxyl is chiral due to the presence of the stereogenic center in the alkyl moiety (Fig. 1) and consists of a pair of enantiomers, with fungicidal activity almost entirely originating from the (−)-(*R*)-enantiomer [3]. In some countries, racemic metalaxyl has been replaced by metalaxyl-M, consisting of >97% of the (−)-(*R*)-enantiomer, thus allowing reduction of application rates and of potential environmental damage [5].

Metalaxyl can induce bradycardia in rats [6,7] and affect the activity of monoamine oxidase in the rat heart [8]. Some data indicates that metalaxyl has activity as a cotoxin or cocarcinogen [9], and can cause chromosomal changes in humans and animals [10].

The metabolism of metalaxyl and metalaxyl-M in animals has been reported [11,12]. Metabolism is rapid with more than 60% of the administered dose excreted within 24 h. Metabolism proceeds through several steps, leading to glucuronic acid conjugates via methyl ester cleavage and benzyl methyl oxidation. Stereoselective kinetic studies, however, have not been reported. This research was conducted to determine kinetic stereoselectivity of the two metalaxyl enantiomers in rabbits with the aim of improving metalaxyl safety to human and animals and minimizing contamination of the environment.

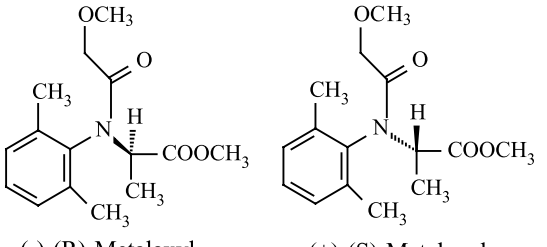

Fig. 1. Chemical structures of metalaxyl enantiomers.

2. Materials and methods

2.1. Chemicals and reagents

Racemic metalaxyl (purity >96%) and pure (−)-(*R*)-metalaxyl (purity >97%) were provided by Institute for Control of Agrochemicals, Ministry of Agriculture (ICAMA) China. Ethyl acetate (Analytical grade) and methanol (HPLC grade) were purchased from Beijing Analytical Reagent Plant (China). Water was purified by a Milli-Q system.

Stock solutions of all analytes, racemic metalaxyl were prepared in methanol. The solutions were stored in capped test tubes at −20 °C. Working standard solutions of (−)-(*R*)- and (+)-(*S*)-metalaxyl (5–5000 μg/ml) were obtained by dilution of the stock solutions in methanol.

2.2. HPLC analysis

Chromatography was performed using an Agilent 1100 series HPLC equipped with a G1311A pump, G1322A degasser, G1328A injector, a 10-μl sample loop, and G1314A VWD. The signal was received and processed by an Agilent Chemstation for LC 3D.

Enantiomers were separated on cellulose–Tris-(3,5-dimethylphenylcarbamate) (CDMPC)-based chiral stationary phase in reversed phase condition. The CSP was synthesized and packed into a 250 × 4.6-mm (i.d.) stainless steel column [13]. The mobile phase was a mixture of 55% methanol and 45% water; flow rate was 1.0 ml/min. Chromatographic separations were conducted at room temperature and VWD detection at 220 nm. The metalaxyl peak was split into (−)-(*R*)- and (+)-(*S*)- enantiomers, and elution order was determined by comparison with the retention time of a pure (−)-(*R*)-isomer standard.