Uludag Univ. J. Fac. Vet. Med.
30 (2011), 1: 47-52

Ecopharmacokinetics of Tetracyclines and Sulfonamides in Soil

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Geliş Tarihi: 08.06.2011
Kabul Tarihi: 11.07.2011

Abstract: The aims of this study were to evaluate the elimination of oxytetracycline, chlortetracycline, sulfadiazine and sulfamethoxazole from soil, and to investigate the influence of pH and the rainfall on elimination of the antibiotics. To detect antibiotic concentrations, leachate samples were collected from soil columns. The antibiotics were extracted by solid phase extraction and analysed by high performance liquid chromatography system. To determine elimination half-life of the antibiotics from soil and relationship between measured parameters, first-order kinetics and statistical analyses were applied to all collected data. Half-life of sulfadiazine and sulfamethoxazole in leachate waters 19 days after treatment was 0.24 hour and this was increased up to 2.25 hours sulfamethoxazole and 3.57 hours sulfadiazine 33 days after treatment. A half-life for tetracyclines was not detected, since there was not consistent trend for the transport of tetracyclines between soil and water compartments. The results of regression analyses showed that the concentrations of antibiotics in the same group changed at the same rate. The pH of leachate samples was positively influenced by increases in rainfall and days after treatment. A significant relationship was not found between the antibiotic concentrations and both pH and rainfall.

Key Words: Ecopharmacokinetics, tetracyclines, sulphonamides, soil.

Toprakta Tetrasiklin ve Sulfonamidlerin Ekofarmakokinetiği

Özet: Bu çalışmanın amacı oksitetrasiklin, klortetrasiklin, sulfadiazine ve sulfametoksazolun topraktan eliminasyonunu incelemek ve pH ile yağışın antibiyotiklerin eliminasyonuna etkisini araştırmaktır. Antibiyotik miktarlarını belirlemek için toprak kolonlarından sızıntı suyu örnekleri toplandı. Antibiyotikler katı faz ekstraksiyon yöntemiyle ekstrakte edildi ve yüksek performanslı sıvı kromatografi ile analiz edildi. Antibiyotiklerin topraktan yarı-ömrünü ve ölçülen parametreler arasındaki ilişkiyi belirlemek için toplanan tüm verilere birinci derece kinetiği ve istatistiksel analiz uygulandı. Sulfadiazin ve sulfametoksazolun uygulamadan 19 gün sonra sızıntı sularındaki yarı-ömrü 0.24 saatti ve bu süre uygulamadan 33 gün sonra sulfametoksazol için 2.25 saate, sulfadiazin için 3.57 saate kadar arttı. Tetrasiklinlerin toprak ve su kompartmanları arasında tutarlı bir geçişi olmadığından tetrasiklinler için bir yarı-ömür tespit edilemedi. Regresyon analizi sonuçları aynı gruptaki antibiyotik miktarlarının aynı oranda değiştiğini gösterdi. Sızıntı suyu örneklerinin pH'sı yağış ve uygulama süresinden pozitif şekilde etkilendi. Antibiyotik miktarları ile pH ve yağış arasında önemli bir ilişki bulunamadı.

Anahtar Kelimeler: Ekofarmakokinetik, tetrasiklinler, sulfonamidler, toprak.

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Introduction

Veterinary pharmaceuticals may be excreted after being used as therapeutics, so the parent compound and/or metabolites then enter the soil due to the spreading of manure and slurry on agricultural land[10,19,24]. Following soil contamination, these compounds may be transported to streams and rivers via runoff and drain flow to groundwater by leaching, and they may also enter into the food chain [2, 5, 12].

Initial steps in the degradation process in the environment involve modification of the active sites of the pharmaceutical compounds. Similar to pharmacokinetics in clinical practice, the concentration of a pharmaceutical compound can be determined in a particular environment and the duration it takes for the compound to be reduced to acceptable levels can be established. Pharmacokinetics in the environment includes all issues mentioned above and is called as eco- or enviropharmacokinetics[11].

The mobility of pharmaceuticals in the environment is largely dependent on the aqueous flow patterns and is counteracted by physical and chemical factors such as sorption, presence of inorganic compounds, pH, and permeability of the respective environmental matrices[11]. For example, tetracyclines (TCs) form complexes with chelating agents, such as polyvalent cations and β-diketones, and strongly bind to proteins and silanol groups[6,15,20]. The sorption behaviour of sulphonamides (SAs) may be influenced by pH, accessible functional groups at organic-mineral surfaces, three-dimensional structure of soil organic matter and the polyvalent and silt contents of soil[4,6,14,16,21,22].

To evaluate the elimination of veterinary antibiotics from soils, various sizes of soil columns were used in previous studies[12,13,18,23]. The elimination is that the concentration of chemical remaining will theoretically never reach zero, and these many half-life cycles would have eliminated about 99% of the compound, which for practical purposes is considered as complete elimination[11]. The aims of this study were to evaluate the elimination of oxytetracycline (OTC) chlortetracycline (CTC), sulfadiazine (SDZ) and sulfamethoxazole (SMZ) from soil by monitoring their residues in leachate waters, and to investigate the influence of pH and the rainfall on elimination of the antibiotics.

Materials and Methods

Chemicals

All antibacterial standards used in the analysis were purchased from Riedel-de Haën. All chemicals used for the extraction of antibacterials from soil samples and solvents used for HPLC-PDA analysis of antibacterials were of analytical grade and purchased from Merck and Sigma-Aldrich.

Experimental Setup and Sampling

In this study, the transport of study compounds in two packed soil columns was investigated. The soil materials were collected from the upper layers of one organic agricultural land, since organic agricultural lands are uncontaminated fields with antibiotics or further chemicals.

Glass columns with an inner diameter of 20 cm and a height of 70 cm were used for the experiments. The glass columns were packed with air-dried and sieved (< 2 mm) soil. Soil used for packing of the columns was characterized for texture, organic carbon content and pH. Semi-liquid manure was fortified with study compounds by adding the powder form of each compound to manure (250 mg/kg manure). Finally, antibiotic-contaminated manure was mixed with top portion of soil in the column. The columns were inserted in an agricultural field and exposed to the natural weather conditions. All experiments were done in spring seasons.

Leachate samples were collected over 129 days and the number of collected leachate samples from the TC column (C_{TC}) and the SA column (C_{SA}) was 17 and 20, respectively. Leachate pH measurements were made immediately after sample collection using a digital pH meter (Sartorius PB 20 AG Göttingen, Germany).

Determination of Antibiotics in the Leachate Samples

The study compounds were extracted from the leachate samples by solid phase extraction using the method described by Blackwell et al.[3]. Briefly, extraction buffer (0.1 M EDTA/0.2 M citric acid/0.4 M Na_2HPO_4/H_3PO_4 100:60:40:2 v/v) and methanol per 400 ml of water were added to each sample. The samples were then cleaned and pre-concentrated by solid phase extraction (SPE) using preconditioned Isolute SAX anion exchange cartridges (6 ml-

500 mg) and Water Oasis HLB polymer cartridges (6 ml-200 mg) in tandem at a flow rate of 10 ml/min. The HLB cartridges were washed sequentially with washing buffer (prepared by diluting the extraction buffer 20-fold), 0.1 M sodium acetate, distilled water and 20% methanol. They were then air dried for 10 minutes, and the extracts were eluted twice 1 ml of methanol. The extracts were stored at -20ºC until analysis.

The soil extracts were analysed by HPLC-PDA using a Shimadzu LC-20A System (Shimadzu) with an octadecylsilane (ODS) (150 mm x 4.6 mm x 5 µm, GL Sciences Inc.). The study compounds were analysed simultaneously. A gradient elution was carried out over 15 min with 0.1% formic acid in acetonitrile (Solvent A) and 0.1% formic acid in water (Solvent B). The initial percent of Solvent A was 5%, which was then increased to 30% from 0 to 6 min and remaining at 30% from 6 to 7.5 min. The percentage of Solvent A was returned to 5% from 7.5 to 10 min and remained at 5% from 10 to 15 min. The flow rate was 0.70 ml/min throughout, and simultaneous detection was performed at 360 nm for TCs and 274 nm for SAs.

Calculation of Elimination Half-Life and Statistical Analysis

First-order kinetics is the simplest kinetic situation in drug metabolism as a typical pharmaceutical compound concentration over time relationship shows this type of kinetics, decreasing at a rate that is proportional to the amount of compound remaining, but never appearing to reach zero. The shape of this relationship is also synonymous to that of the concentration of drug at the absorption site. The half-life of a drug in the environmental matrix ($t_{1/2}$) can be calculated from Equation 1[11].

$$t_{1/2} = 0.693/k_e \quad \text{(Eq. 1)}$$

$$k_e = \text{Slope} = \Delta Y/\Delta X \quad \text{(Eq. 2)}$$

The k_e is the elimination of degradation rate constant represented by the slope of the line on the log concentration versus time curve (Equation 2).

Correlation and regression analyses were used to examine the relationship between measured parameters.

Results and Discussion

Measured physical and chemical properties of the soil were given in Table 1.

Table 1. Physical and chemical properties of the soil

Tablo 1. Toprağın fiziksel ve kimyasal özellikleri

Soil Properties	Unite	Soil
Clay (<0.002 mm)	(% mass)	45.84
Silt (0.002-0.05 mm)	(% mass)	24.65
Sand (0.05-2.000 mm)	(% mass)	29.51
C_{org}	(% mass)	1.31
pH (0.01 M $CaCl_2$)		7.74

The pH of the leachate samples from the C_{TC} and C_{SA} were in the range of 7.01-8.10 and 6.42-8.20, respectively. The means of pH values were 7.64 (C_{TC}) and 7.79 (C_{SA}). Total rainfalls over the period of the studies were 57.62 mm (C_{TC}) and 86.38 mm (C_{SA}).

Recoveries and Concentrations of Antibiotics in Leachate Samples

Extractions were performed in triplicate and the collected data were used to calculate the recovery values, which are given in Figure 1. The standard-based limit of detections (LODs), defined as the concentration of standards used for the calibration in the peak area with a signal-to-noise ratio (S/N) of 3.3, were 10 (OTC), 35 (CTC) and 25 µg/L (SDZ and SMZ).

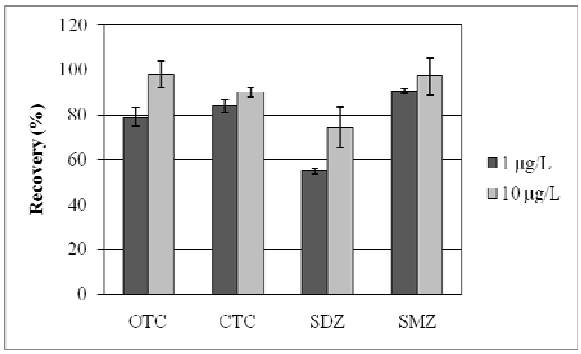

Figure 1. *Recovery rates of tetracyclines and sulfonamides in leachate samples*

Şekil 1. *Sızıntı suyu örneklerindeki tetrasiklin ve sulfonamidlerin geri kazanım oranları*

The first leachate sample from the C_{TC} was collected 16 days after treatment (DAT), and the last one was collected 113 DAT. OTC was not detected in only one sample collected 19 DAT, and OTC concentration in the other leachate samples was 470 to 4885 µg/L. CTC was detected in only nine leachate samples collected from the C_{TC} and its concentration was

218 to 2987 µg/L. SDZ and SMZ could be detected in all of the collected samples from the C_{SA}. The concentrations of SDZ and SMZ 19 DAT were in the range of 66.27-610.88 µg/L (Figure 2) and 108.75-672.94 µg/L (Figure 3), respectively. In the period after the first two-peak detection of both antibiotics, the concentrations of the antibiotics were below 20 µg/L. SDZ and SMZ were still detected in leachate samples 129 DAT at concentrations of 1.80 µg/L and 1.42 µg/L, respectively.

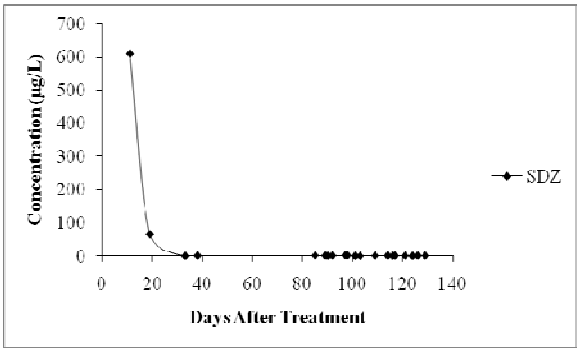

Figure 2. *Concentrations of sulfadiazin in leachate waters during the experimental period*

Şekil 2. *Deney süresince sızıntı sularındaki sulfadiazin miktarları*

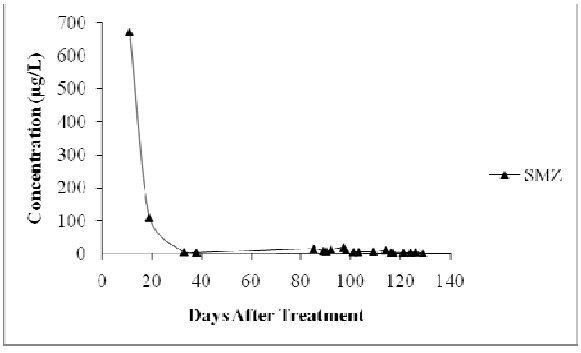

Figure 3. *Concentrations of sulfametoksazol in leachate waters during the experimental period*

Şekil 3. *Deney süresince sızıntı sularındaki sulfametoksazol miktarları*

Elimination Half-Life and Statistics

Half-life of SDZ and SMZ in leachate waters 19 DAT was 0.24 hour and this was increased up to 2.25 hours (SMZ) and 3.57 hours (SDZ) 33 DAT. A half-life for TCs was not detected, since there was not consistent trend for the transport of TCs between soil and water compartments.

The significant correlations for TCs and SAs at the 0.01 and 0.05 levels were given in Table 2.

Table 2. Correlation matrix for tetracyclines and sulphonamides

Tablo 2. Tekrasiklin ve sulfonamidler için korelasyon matriksi

		pH	Rainfall	DAT	OTC	CTC
TCs	pH	-				
	Rainfall	0.601*	-			
	DAT	0.794**	0.607*	-		
	OTC	ns	ns	ns	-	
	CTC	ns	-0.728*	ns	0.762*	-
		pH	Rainfall	DAT	SDZ	SMZ
SAs	pH	-				
	Rainfall	ns	-			
	DAT	ns	ns	-		
	SDZ	ns	ns	-0.403*	-	
	SMZ	ns	ns	-0.680**	0.727**	-

ns: Not significant

*: Correlation is significant at the 0.05 level

**: Correlation is significant at the 0.01 level

For TCs, significant regression equations were found between OTC and CTC, pH and DAT, pH and rainfall (Equations 3-5). For SAs, significant regression equation was found between only SDZ and SMZ (Equation 6).

$\log OTC = 0.68 \times \log CTC + 0.27$; $R^2 = 0.70$, $p = 0.010$ (Eq. 3)

$pH = 0.01 \times DAT + 7.15$; $R^2 = 0.59$, $p = 0.001$ (Eq. 4)

$pH = 0.10 \times Rainfall + 7.27$; $R^2 = 0.38$, $p = 0.011$ (Eq. 5)

$SDZ = 0.91 \times SMZ - 6.02$; $R^2 = 1.00$, $p = 0.000$ (Eq. 6)

The results of regression analyses showed that the concentrations of antibiotics in the same group changed at the same rate (Equation 3 and 6). The pH of leachate samples was positively influenced by increases in rainfall and DAT (Equations 4-5). A significant relationship was not found between the antibiotic concentrations and both pH and rainfall.

The concentration of the study compounds applied to the columns (250 mg/kg) is much higher than concentrations detected in the agricultural fields in Turkey (approx. 0.100 mg/kg). De Liguoro et al.[8] found that the concentration of OTC in undiluted cattle manure can reach 872 mg/kg. The relatively high concentration was therefore used in the current study.

The mean value of CTC concentrations in the leachate samples was 981 µg/L, which was lower than the mean value (1829 µg/L) of OTC concentrations. In addition, CTC could be detected in only 53% of all leachate samples, while OTC was found in 94% of all leachate samples. Therefore, OTC might be a main contaminant of soils due to its high delectability. Indeed, Cengiz et al.[6,7] stated that OTC is greatly responsible for antibiotic pollution in the agricultural fields. However, Blackwell et al. [1] could not detect any OTC residue in leachate samples collected from a lysimeter over three months, despite using a similar size (60 cm long and 24 cm in diameter) lysimeter to one used in the current study. This variability may be a consequence of the difference between the characteristics of soils used in the studies or higher antibiotic concentration used in the current study.

In this study, concentrations of SDZ and SMX were dramatically reduced to 1.01 µg/L and 5.41 µg/L 33 DAT and in some leachate samples those re-increased up to 2.88 µg/L and 18.70 µg/L, respectively. The remaining percentages of initially detected concentrations were 0.4% (SDZ) and 0.2% (SMX). The remaining percentages and the duration need to be reduced to the lowest detected concentrations of the compounds in leachate samples, a conclusion consistent with previous studies[1,12]. Most sulfonamides are positively charged under acidic conditions, neutral between pH 2.5 and 6, and negatively charged at alkaline conditions[9]. Therefore, under typical environmental pH conditions (pH 7-8), sulfonamides are negatively charged (95-100%;[17]), which can increase their transport velocity in porous media. Thus, the possibility for detecting sulfonamides in aquatic environment increases. Manure application caused slightly an increase in the pH values of leachate waters, but pH mostly remains at level of typical environmental pH values (pH 7-8) or became slightly alkaline that alkaline pH values can increase sulfonamide' transport to aquatic phase.

In conclusion, statistical analyses have shown that the concentrations of antibiotics in the same group change at the same rate. Rainfall and pH changes in the range of 6-8 have not an impact on the transport of TCs and SAs between soil and water.

Acknowledgement

The financial support for this study from the Research Fund of Uludag University (V-2006/56) is gratefully acknowledged.

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